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FURTHER OBSERVATIONS UPON THE COMPLEMENT FIXATION TEST IN THE DIAGNOSIS OF AMEBIASIS*

AN ANALYSIS OF THE RESULTS OF THE TEST IN ONE THOUSAND INDIVIDUALS

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IN 1927,[1] I demonstrated that the blood serum of individuals infected with Endamoeba histolytica, the cause of amebic dysentery, contained complement fixing bodies, and in subsequent contributions[2, 3, 4, 5, 6] described the technic and results of a complement fixation test which I devised for use in the diagnosis of this condition. In the present contribution the results obtained with this test as applied to one thousand individuals will be described together with a consideration of the value of the test in diagnosis and in the control of the treatment of amebiasis.

Historical Summary.—In 1914, Izar,[7] using aqueous extracts of fecal material containing Endamoeba histolytica and liver abscess pus obtained from infected kittens as antigens, obtained complement fixation in the blood serum of 5 individuals infected with this parasite and 3 kittens. In 1920[8] von Hage repeated Izar's work but could not confirm it. In 1921[9] Scalas, using as an antigen an aqueous extract of mucous flakes from feces containing Endamoeba histolytica, obtained positive results in cases of amebic dysentery. No other publications upon the occurrence of complement fixation in amebiasis appeared until mine in 1927.

Since my observations were published the occurrence of complement fixation in amebiasis has been confirmed by several observers, notably Spector, Fernandez, Heathman, and Sherwood and Heathman. Spector[10] using a technic practically identical with mine obtained positive results in cases of amebiasis; Fernandez[11] immunized rabbits by injection of extracts of Endamoeba histolytica and demonstrated the presence of complement fixing bodies in

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the blood serum of these animals; Heathman[12] was also successful in demonstrating complement fixing bodies in the blood serum of rabbits immunized by injections of extracts of Endamoeba histolytica, while Sherwood and Heathman[13] obtained complement fixation in the blood serum of cases of amebic dysentery.

Material.—The observations detailed in this contribution are based upon the results of the complement fixation test upon the blood serum of 1000 individuals suffering from definite symptoms of amebiasis; amebic "carrier" cases having no symptoms of the infection; patients in the wards of a general hospital suffering from other disease conditions than amebiasis; and patients sent to me for examination or seen by me in consultation. In very many of these cases repeated complement fixation tests were made upon the same individual, so that in reality the observations here recorded are based upon the results of at least 2000 complement fixation tests which have been controlled by examinations of the feces for Endamoeba histolytica.

Technic.—The technic employed by me has not differed from that previously described,[14] to which the reader is referred. A human hemolytic system has been used, the sera to be tested have been inactivated by heating at 56° C. in the water-bath for one-half hour, and the antigen employed has been an alcoholic extract of forty-eight-hour old cultures of Endamoeba histolytica grown upon the Boeck-Drbohlav medium. The test was not considered as positive unless a three- or four-plus reaction was obtained on a four-plus scale.

Results.—This contribution deals only with the complement fixation test in those individuals in whom the results have been checked by an examination of the feces for Endamoeba histolytica. To date, 1000 such individuals have been tested, of which 175, or 17.5 per cent gave a positive result and 825, or 82.5 per cent gave a negative result. It should be remembered that the vast majority of the individuals examined were patients in hospital and that many of them were soldiers who had served in localities where amebic infection was common, thus accounting for the comparatively high incidence rate of 17.5 per cent.

Of the 175 individuals giving a positive result, an examination of the feces resulted in the demonstration of the presence of Endamoeba histolytica in 157, or 89.7 per cent, while in 18, or 10.2 per cent, the parasite could not be demonstrated in the feces. In justice to the test it should be stated that it was impossible to make more than one or two examinations in the cases negative for the parasite. It is well known that in some cases as many as six examinations must be made of the feces before one is justified in stating that a patient is negative for Endamoeba histolytica and that these examinations must be made at intervals of at least two days, as observation has shown that this parasite is frequently impossible of demonstration in the feces at certain times, although it may be present in large numbers at other times. It is very probable that some of these cases would have proved positive for the parasite had a greater number of examinations been made.

Of the 175 individuals giving a positive reaction a record was kept of the occurrence of other intestinal protozoa in 110 cases. Of these, 66 were

infected with Endamoeba histolytica alone; 14 with Endamoeba histolytica and Endamoeba coli; 9 with Endamoeba histolytica and Endolimax nana; 3 with Endamoeba histolytica, Endolimax nana and Endamoeba coli; 10 with Endamoeba histolytica and Chilomastix mesnili; 5 with Endamoeba histolytica and Giardia lamblia and 3 with Endamoeba histolytica and Trichomonas hominis.

Of the 18 cases in which Endamoeba histolytica was not found in the feces, 7 were in individuals having indefinite intestinal symptoms while in 11 a clinical diagnosis of chronic ulcerative colitis had been made.

There were 835 individuals tested who gave a negative reaction, and of these Endamoeba histolytica was found in the feces of 12, or 1.4 per cent. Three of these cases were diagnosed acute amebic dysentery; four were diagnosed chronic ulcerative colitis; one, amebic abscess of the liver; three had indefinite intestinal symptomatology and one was symptomless. It is thus evident that not all individuals infected with Endamoeba histolytica give a positive reaction with the complement fixation test but the very small percentage that do not, in our experience, is noteworthy.

Many of the negative individuals were infested with other species of intestinal protozoa. Thus, of 676 who gave a negative reaction no less than 220, or 32.5 per cent, were positive for one or more species of intestinal protozoa other than Endamoeba histolytica. One hundred, or 14.7 per cent, were infested with Endamoeba coli; 64, or 9.9 per cent, with Endolimax nana; 3, or 0.4 per cent, with Iodamoeba williamsi; 26, or 3.8 per cent, with Chilomastix mesnili; 16, or 2.3 per cent, with Trichomonas hominis; and 11, or 1.6 per cent, with Giardia lamblia. There were 14 mixed infections with Endamoeba coli and Endolimax nana; 6 with Endamoeba coli and Chilomastix mesnili; 4 with Endamoeba coli and Giardia lamblia, and 3 with Endamoeba coli and Trichomonas hominis.

That the infestation of the human intestine with species of amebae other than Endamoeba histolytica does not give rise to a positive complement fixation reaction is proved by the fact that of 700 individuals giving a negative reaction, including the 676 mentioned above, no less than 176, or 25.1 per cent, were infested with some other species of intestinal ameba.

Approximately two-thirds of the 825 individuals giving a negative reaction with the complement fixation test were patients in a large general hospital, or from clinics, and were suffering from many acute and chronic disease conditions. The remaining third were normal individuals used as controls. It is thus evident that this test does not give positive results in normal individuals or in those suffering from other disease conditions than amebiasis, with the possible exceptions discussed later.

The Specificity of the Reaction.—As pure cultures of Endamoeba histolytica are not yet available, the antigenic extracts used in this test contain extractives of both this parasite and the bacteria growing in culture with the amebae. Alcoholic extracts of these bacteria, grown upon the Boeck-Drbohlav medium, and prepared in the same manner as the antigen used in the test have been used as controls and in no instance have such extracts given positive

results, so that it is evident that the positive results obtained with the ameba antigen are actually specific and are not due to the bacterial extractives contained in the antigen. That this is true is evidenced by the disappearance of the complement fixation reaction after proper treatment of the amebic infection and the disappearance of Endamoeba histolytica from the feces. It has invariably been our experience that the complement fixation reaction disappears after treatment resulting in the disappearance of the amebae from the feces and this is well illustrated in Table I, which gives the time of disappearance of the reaction after treatment in 30 cases in which this could be accurately ascertained.

TABLE I

ILLUSTRATING THE TIME OF DISAPPEARANCE OF THE COMPLEMENT FIXATION TEST FOR ENDAMOEBA HISTOLYTICA AFTER TREATMENT

CASE	RESULT OF TEST BEFORE TREATMENT	FECES EXAMINATION FOR E. HISTOLYTICA BEFORE TREATMENT	RESULT OF TEST AFTER TREATMENT	FECES EXAMINATION FOR E. HISTOLYTICA AFTER TREATMENT	DAYS AFTER CESSATION OF TREATMENT
1	++++	Positive	–	Negative	3
2	++++	Positive	–	Negative	3
3	++++	Positive	–	Negative	4
4	++++	Positive	–	Negative	5
5	++++	Positive	–	Negative	5
6	++++	Positive	–	Negative	6
7	+++	Positive	–	Negative	7
8	++++	Positive	–	Negative	9
9	++++	Positive	–	Negative	11
10	+++	Positive	–	Negative	12
11	++++	Positive	–	Negative	14
12	++++	Positive	–	Negative	14
13	++++	Positive	–	Negative	14
14	++++	Positive	–	Negative	14
15	++++	Positive	–	Negative	14
16	++++	Positive	–	Negative	14
17	++++	Positive	–	Negative	14
18	++++	Positive	–	Negative	14
19	++++	Positive	–	Negative	14
20	++++	Positive	–	Negative	14
21	++++	Positive	–	Negative	14
22	++++	Positive	–	Negative	14
23	++++	Positive	–	Negative	14
24	++++	Positive	–	Negative	14
25	++++	Positive	–	Negative	14
26	++++	Positive	–	Negative	18
27	++++	Positive	–	Negative	21
28	++++	Positive	–	Negative	21
29	++++	Positive	–	Negative	21
30	++++	Positive	–	Negative	28

Upon reference to Table I it will be noted that the reaction disappeared in 2 cases, three days after the cessation of treatment; in 1 case, four days afterward; in 2 cases, five days afterward; in 1 case each, six, seven, nine, eleven, twelve, eighteen, and twenty-eight days after cessation of treatment respectively; in 15 cases, fourteen days afterward; and in 3 cases, twenty-one days after cessation of treatment. It is evident that the time of disappearance of the reaction after treatment varies considerably in different individuals as would be expected, but that in the vast majority of the cases, as shown

in Table I, the reaction had disappeared by the end of the second week after the cessation of specific treatment and the disappearance of Endamoeba histolytica from the stools. Thus, of the 30 cases studied, the reaction had disappeared within fourteen days in no less than 25 or 86.6 per cent, while in the remainder it had disappeared by the twenty-eighth day after the cessation of treatment. The disappearance of the reaction after treatment resulting in the elimination of Endamoeba histolytica demonstrates, in my opinion, the specificity of this complement fixation test for infection with Endamoeba histolytica. It may be stated that similar results are obtained in experimental amebiasis in dogs, as recently reported by me.[15]

The only conditions in which apparently false positive reactions have been obtained with this test are syphilis and chronic ulcerative colitis. Of 110 individuals giving a positive complement fixation test for infection with Endamoeba histolytica, 15, or 13.6 per cent, also gave a positive Wassermann and Kahn test, while of 676 individuals giving a negative reaction for Endamoeba histolytica, 56, or 8.2 per cent, gave a positive Wassermann or Kahn test. Of the 15 individuals giving a positive complement fixation test for Endamoeba histolytica as well as a positive Wassermann and Kahn test, 9 failed to show Endamoeba histolytica in the feces. Because of the greater number of cases giving a positive reaction with all three tests and the failure to find Endamoeba histolytica in over half of such cases, I have stated[16] that "in rare instances, patients suffering from syphilis may give a positive result with this test." However, further observations have convinced me that this is not true and that syphilis does not give a reaction with the complement fixation test for amebiasis. This conclusion is based upon the observation that in properly treated cases positive with the three tests, the complement fixation reaction disappears after the elimination of Endamoeba histolytica while the Wassermann and Kahn reactions persist, and that the apparent excess of positive Wassermann and Kahn reactions in individuals giving a positive complement fixation test for amebiasis disappears as a larger number of individuals infected with this parasite have been tested. At the present time it is not believed that syphilis gives a positive result with this test unless there is a coincident infection with Endamoeba histolytca.

The question of the occurrence of a positive complement fixation reaction with this test in certain cases of chronic ulcerative colitis is an interesting and important one. In 1932, Kiefer,[17] of the Lahey Clinic, reported the results of this test in 19 cases diagnosed as chronic ulcerative colitis of which 15 gave a positive result. Of the 15 positive cases Endamoeba histolytica could be demonstrated in only 3 cases. The complement fixation tests in this series of cases were made for Dr. Kiefer by me, while the stool examinations were made in Boston by competent parasitologists. In his paper, Kiefer states that cases of diarrhea caused by functional disturbance, also tested by me, gave consistently negative results, and that the large percentage of positive results in chronic ulcerative colitis remains unexplained and suggests some relationship between this condition and amebic infection. He suggested that chronic ulcerative colitis may be "a pyogenic infection of the colon superimposed upon an original amebic ulceration."

In my personal experience, exclusive of the tests made for Dr. Kiefer, there have been eleven cases diagnosed as chronic ulcerative colitis which have given a positive reaction with the complement fixation test in which the examination of the feces for Endamoeba histolytica resulted negatively. In all of these cases it was impossible to make more than one or two examinations of the feces, so that it is impossible to be sure that the parasite was really absent, for in many other cases diagnosed chronic ulcerative colitis in which the reaction was positive, repeated examinations had to be made in order to demonstrate the parasite. In several such cases as many as six to eight examinations of the feces were made before Endamoeba histolytica was found and, even then, in small numbers. In some cases daily examinations were made for over a week and the parasite found at the last examination, so that unless at least six examinations are made upon different days it is impossible to be sure that Endamoeba histolytica is not present. In view of this experience I am convinced that this test does not give a positive result in cases of chronic ulcerative colitis unless Endamoeba histolytica is present. This conclusion is further justified by the results of antiamebic treatment in these cases, for Kiefer states that treatment with chiniofon of the cases of chronic ulcerative colitis giving a positive reaction with the complement fixation test, but negative for Endamoeba histolytica, resulted in the disappearance of the symptoms in 4 cases with no return for from ten to fourteen months; a definite improvement in 5 cases, while in the remaining the treatment had not been completed and concludes that antiamebic treatment should be tried in all cases of chronic ulcerative colitis. My experience agrees with that of Kiefer as regards the specific effect of antiamebic treatment in cases of ulcerative colitis giving a positive complement fixation test, but negative for Endamoeba histolytica, for all cases so treated have been benefited or apparently cured.

The theory of Kiefer that chronic ulcerative colitis may be a pyogenic infection engrafted upon an original amebic infection is worthy of serious consideration and study. It would explain the occurrence of a positive complement fixation test in some cases of this condition in which the parasite cannot be demonstrated in the feces, for in such cases there may be a localization of the organisms in the tissues of the intestine with few or none occurring in the lumen of the gut, the conditions there being unfavorable for the propagation of the amebae, owing to the presence of the pyogenic infection. In time, the amebic infection in the tissues would disappear and in such cases the complement fixation reaction will result negatively, as it does in many cases diagnosed as chronic ulcerative colitis, in my experience. At the present time it is believed that the occurrence of a positive reaction with the complement fixation test for amebiasis in certain cases of chronic ulcerative colitis indicates the presence in the tissues of the colon of Endamoeba histolytica and that when this parasite is absent positive reactions will not be obtained in this condition.

With the exception of the conditions discussed there is no evidence that the complement fixation test for amebiasis gives positive results in either normal individuals or those suffering from other diseases.

The Value of the Test in Diagnosis.—Continued observations with this test have convinced me of its value in the diagnosis of amebiasis. Where the services of a trained protozoologist are available, and it is possible to make repeated microscopic examinations of the feces in suspected cases, this test would be of comparatively little help in diagnosis, as I am convinced that every case of intestinal infection with Endamoeba histolytica can be diagnosed by microscopic examinations of the feces, provided repeated examinations are made, if necessary. However, the test has proved of value in my hands in the discovery of cases of amebic abscess of the liver in patients whose feces were negative for the parasite in four instances, and in several other cases the routine use of the test has resulted in the discovery of liver abscesses due to Endamoeba histolytica, in which subsequent fecal examinations demonstrated the presence of the parasite, although no symptoms of amebic infection of the intestine were present. The routine application of the test has also given positive results in scores of cases where an amebic infection was not suspected and where the symptoms were atypical or so slight as to be overlooked by the clinician. The discovery and proper treatment of such infections is of the greatest importance from both a therapeutic and prophylactic standpoint. I[18] have recently reviewed the evidence demonstrating that marked ulcerative lesions due to Endamoeba histolytica may exist in the intestine of individuals who have shown no clinical evidence of amebic infection, so that absence of symptoms does not prove that amebic infection is absent or that definite pathologic lesions of the intestine may not exist, and the complement fixation test for amebiasis should prove of the greatest service as an aid in the recognition of such cases of latent amebiasis.

The use of the test as *a control of treatment* should also prove of definite value. As already stated, proper treatment followed by the disappearance of Endamoeba histolytica from the feces, is also followed by the disappearance of the positive complement fixation reaction, and, in cases which have relapsed, by a return of the positive reaction. It is thus possible to determine the effect of treatment with any particular drug and the relative efficiency of various methods of medication by repeated complement fixation tests. In some cases in which a relapse occurred a return of the positive complement fixation reaction has been noted even before the amebae could be demonstrated in the feces, thus indicating a multiplication of the organisms in the tissues of the intestine sufficient to produce such a reaction before they become numerous enough in the lumen of the bowel to be demonstrable in the feces.

At the present time the practical application of the test is limited by the difficulty of preparing an efficient antigen, and the small amounts that can be obtained from large numbers of cultures of Endamoeba histolytica. It is hoped that, in the near future, improved methods of preparing antigens will make it possible for the test to be more generally used in clinical laboratories than it is at present.

CONCLUSIONS

1. Specific complement fixing bodies may be demonstrated in the blood serum of individuals infected with Endamoeba histolytica when alcoholic extracts of cultures of this parasite are used as antigens.

2. Normal individuals or those suffering from other infections or diseases do not give a positive complement fixation reaction with such antigens, unless there is a coincident infection with Endamoeba histolytica.

3. The specific complement fixing bodies disappear from the blood serum following antiamebic treatment and the disappearance of Endamoeba histolytica from the feces.

4. In relapsing cases of amebiasis the complement fixation test, which has been negative during the interval of apparent freedom from Endamoeba histolytica, again becomes positive, in rare instances even before the parasite is again demonstrable in the feces. Thus, a negative reaction, unless repeatedly so for several weeks, does not prove the absence of amebic infection or that antiamebic treatment has resulted in cure.

5. The time of disappearance of the positive complement fixation reaction after treatment resulting in the disappearance of Endamoeba histolytica from the feces has varied, in this series of observations, between three and twenty-eight days. In 86.6 per cent of the cases studied the reaction disappeared within fourteen days after the cessation of antiamebic treatment, and in 96.6 per cent within twenty-one days after cessation of treatment resulting in the disappearance of the parasite from the feces.

6. Individuals infested with other species of amebae, or with the intestinal flagellates, do not give a positive complement fixation with this test unless Endamoeba histolytica is also present.

7. The complement fixation reaction occurs in all stages of amebiasis but the strongest positive results are usually obtained in symptomless "carriers" or in those presenting mild symptoms of infection with Endamoeba histolytica. In several cases of very acute amebic colitis the reaction has been doubtful or negative, although in most severe cases the reaction has been positive.

8. The test has proved of value in the diagnosis of cases of amebic abscess of the liver unaccompanied by intestinal symptoms; in the diagnosis of apparently healthy "carriers" of Endamoeba histolytica and of those presenting atypical or mild symptoms of infection; and in the control of antiamebic treatment.

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RESULTS OF TREATMENT IN RHEUMATOID ARTHRITIS WITH REFERENCE TO FOCI OF INFECTION AND STREPTOCOCCUS VACCINE*†

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IN RECENT years two forms of therapy have arrested the attention of physicians engaged in the study of rheumatoid arthritis, namely, the removal of foci of infection and the administration of streptococcus vaccine.

As far back as twenty years ago, Billings[1] pointed out the relationship of foci of infection to chronic arthritis. Since that time, certain investigators have enthusiastically advocated the radical removal of such foci in arthritic patients; some have been conservative regarding this procedure; while others have been frankly skeptical. Even today, there seems to be no unanimity of opinion as to the value of removing infected foci in the treatment of this disease.

The other form of therapy which we are considering in this paper, the administration of streptococcus vaccines, has recently enjoyed considerable popularity. Several different types of streptococci have been employed in the preparation of these vaccines, and two methods of administration, the subcutaneous and the intravenous, have been adopted. Regardless of the

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†Rheumatoid arthritis is considered synonymous with chronic infectious arthritis and atrophic arthritis.

type of streptococcus used, or the method of administration, the therapeutic results have generally been reported as favorable.[2]

THE PRESENT STUDY

The present study was undertaken in the hope of crystallizing our own impression as to the relative value of removing different foci of infection in patients with rheumatoid arthritis, as well as to determine the merits of streptococcus vaccine therapy. The patients included in this report were ambulatory and were treated in the Arthritis Department of the Cornell Clinic between 1923 and 1932. As attendance at this clinic requires payment for services, the patients were of moderate means, lived in favorable environments, and were intelligent and cooperative; all resided in the metropolitan area of New York City, and when able they were permitted to pursue their usual occupations. In order to avoid confusion, individuals having more than one type of focus of infection removed were not included in this study. When patients were treated with vaccine only, either foci of infection had not been discovered or had been previously removed.

Forms of treatment supplementary to removal of foci of infection and administration of vaccine were limited to a minimum. As the average patient with rheumatoid arthritis is under-nourished, a generous unrestricted diet, high in vitamin content, was usually recommended. The markedly overweight were given a diet limited in carbohydrates, but they comprised a small proportion of our series. Aspirin and sodium salicylate were frequently prescribed for the relief of pain, and sometimes patients were advised to apply heat to the affected joints, by means of an electric pad, infra-red lamp, or hot compresses. Cod liver oil was given in some cases.

In our experience, patients with well developed rheumatoid arthritis are seldom cured of the disease, if the word *cured* implies complete and permanent relief from all pain, swelling, and stiffness. The few who were discharged as *cured* have usually, at some later period, developed at least vague and transitory symptoms and sometimes suffered complete relapse. For these reasons, we have not classified any of our cases as *cured,* being content to use the word *improved.* Considerable care has been exercised at all times not to confuse temporary changes due to climatic or other varying conditions with more lasting ones. No patient has been included who has not been under observation for at least two months, and many have been followed for several years.

For convenience of study the patients were divided into three groups according to clinical severity. The first group comprises those who complained of pains in the joints, but who failed to show any evidence of arthritis at the time of examination other than tenderness and stiffness of the joints. The second group includes those who presented the typical picture of well developed rheumatoid arthritis with characteristic periarticular swelling of the joints, usually including fusiform fingers. The third group is similar to the second, but comprises patients who were in a more advanced stage of the disease, deformities such as ulnar deviation, and partial or complete ankylosis of

the joints characterize this group. In the tables these three groups have been represented by the symbols +, ++, and +++ respectively.

VACCINES

Preparation of Vaccines.—The various vaccines used in this study were similarly prepared. When several strains were to be included in one vaccine each was treated separately during the early stages of the process. Sufficient growth was obtained by cultivation in beef-heart infusion broth for eighteen to twenty-four hours. After centrifuging the culture, the supernatant broth was discarded, and the sediment washed twice in saline. The bacteria were then suspended in physiologic saline at a concentration of one billion organisms per cubic centimeter and bottled, 0.15 per cent trikresol being added for killing and preserving. After shaking, the bottles were allowed to stand in an inverted position for forty-eight hours. Tests for sterility were then made by culturing the vaccine in blood broth and blood agar.

"Typical Strain" Vaccine.—In a previous article we, in collaboration with Cecil,[3] reported the isolation of streptococci from the blood of a high percentage of patients with rheumatoid arthritis. Of the streptococcus strains thus recovered, 83.3 per cent were similar both culturally and biologically, and were designated as "typical strains." On laboratory mediums, these strains presented the appearance of attenuated hemolytic streptococci. In later papers,[4] it was shown that the serums of a high percentage of patients with well developed rheumatoid arthritis gave strong specific agglutination reactions with "typical strain" streptococci. Because of the apparent etiologic relationships of these organisms to rheumatoid arthritis, they were considered the most suitable for vaccine therapy, and ten of them were used in the preparation of the so-called "typical strain" vaccine. The vaccines were made in large quantities about every three months, the same strains being used each time. Stock cultures of these organisms were maintained in the frozen and dried state as well as in blood broth and blood agar mediums.

Other Streptococcus Vaccines.—Autogenous vaccines were prepared as soon as possible after the streptococci had been isolated, in sufficient quantity to last a patient throughout the course of his treatment. Polyvalent streptococcus vaccines were made from organisms recovered from a wide variety of sources and varied from time to time.

Subcutaneous Administration.—All vaccines for subcutaneous use contained one billion organisms per cubic centimeter. Injections were given at weekly intervals, beginning with 0.1 c.c. and increasing the amount each time until a maximum of 1 c.c. was reached. The treatment was continued at this final level throughout the remainder of the course. Mild local reactions, as indicated by swelling, tenderness, and pain at the site of injection, frequently occurred, but severe local reactions, and generalized reactions as indicated by fever and malaise, were seldom noted. If such reactions did develop, the amount of vaccine was increased more gradually.

Intravenous Administration.—The vaccine for intravenous use was prepared merely by diluting the subcutaneous vaccine with physiologic saline.

Injections were given at weekly intervals, beginning with 50,000 organisms, and increasing the amount at each succeeding inoculation, according to varying rates. For a certain number of patients, the dosage was increased by about 50,000 organisms each time, a single injection never exceeding one million organisms. For others, the rate of increasing the amount injected was more rapid, and at about the tenth injection approximately ten million organisms were being given. Subsequent doses for these patients were then maintained at the ten million level. The dosage for twenty patients in the group receiving "typical strain" intravenous vaccine only and for a few in the group receiving both subcutaneous and intravenous injections was increased by the simple process of doubling the amount of the previous injection until a febrile reaction occurred. Subsequent injections were regulated to a point where the patient developed no more than a mild generalized reaction. Febrile reactions were generally elicited when a patient received an injection containing between one hundred and five hundred million organisms, although one patient who was given a billion organisms at one time had no reaction whatever. Regardless of whether the injections were small or large, the percentage of improvement was similar and, therefore, all of these cases are classified together.

RESULTS OF TONSILLECTOMY

It is often a matter of considerable difficulty to determine whether any particular pair of tonsils is or is not diseased. For this reason, some physicians recommend tonsillectomy for all patients suffering from rheumatoid arthritis, providing their general physical condition is satisfactory. In the present series, however, this policy was not adopted. Tonsils were removed only when it was the opinion of the Nose and Throat Department that they were infected. In making a diagnosis of diseased tonsils, emphasis was placed on a history of previous attacks of sore throat, tonsillitis, and peritonsillar abscess, the size and fixity of the tonsils, the color of the anterior pillars, and the possibility of expressing exudate from the crypts were also taken into consideration.

Altogether, 103 patients were subjected to tonsillectomy and of these, 60, or 58.3 per cent, were improved (Table I). In some patients, the change was immediate; in others, it was gradual, extending over a period of weeks or months, while there were a few cases in which the joint symptoms were temporarily increased, although lasting improvement was eventually noted. Among the unimproved was a small number whose arthritis was apparently worse as a result of the operation. Twenty patients received no therapy other than tonsillectomy. The remaining eighty-three were given in addition some form of streptococcus vaccine, although they in no wise represented a group unimproved by tonsillectomy, the injections being frequently started either before this operation or shortly afterward. In some cases the injections were subcutaneous, in others, intravenous, while certain of the patients

TABLE I

RESULTS OF TONSILLECTOMY

	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
Tonsillectomy only	20	12	60.0
Tonsillectomy and "typical strain" vaccine	57	31	54.4
Tonsillectomy and other streptococcus vaccines	26 (83)	17 (48)	65.4 (57.8)
Total	103	60	58.3

received both. The method of preparation of the vaccine, as well as the manner of increasing the dosage was similar to that used with patients who received vaccine treatment alone. The average number of injections for the patients in this group was 21.2, although there was considerable variation in the individual dosage. Fifty-seven were given "typical strain" vaccine, and of these, 31, or 54.4 per cent, were improved. Twenty-six received various other kinds of streptococcus vaccine, such as autogenous vaccines prepared from hemolytic, green producing and indifferent streptococci, alone or combined. Of these 26 cases, 17, or 65.4 per cent, were improved, but, on careful analysis, no particular merit could be found for any of the types of vaccine employed, and they were, therefore, classified together.

Although the number of patients having tonsillectomy only was small, it was interesting to note that they showed as high a percentage of improvement as those having both tonsillectomy and streptococcus vaccine.

In Table II the cases are arranged according to the severity of the disease. The group having mild arthritis showed an improvement of 66.7 per

TABLE II

RESULTS OF TONSILLECTOMY ACCORDING TO SEVERITY OF DISEASE

SEVERITY OF DISEASE	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
+	27	18	66.7
++	51	30	58.8
+++	25	12	48.0
Total	103	60	58.3

cent; that with well developed signs, 58.8 per cent, and the still more advanced, 48.0 per cent. As would be expected, the mild cases benefited more from tonsillectomy than the severe ones.

Age also played an important rôle in the patient's chances toward recovery (Table III). Of 58 patients forty years of age or under, 39, or 67.2 per cent, were improved, compared to 45 over forty years of age with improvement in only 21, or 46.7 per cent.

The duration of the disease, on the contrary, was apparently an unimportant factor (Table IV). Of those having the disease one year or less, 59.2 per cent were improved; of those having it for more than a year, 57.4 per cent were improved, remarkably similar figures.

RESULTS OF REMOVING ABSCESSED TEETH

In studying the effect on arthritis of the removal of diseased teeth it was considered advisable to include only patients whose teeth showed definite evidence of abscess formation. The diagnosis was largely made from the study of x-rays, and was frequently confirmed by examination of the extracted teeth. Simple pyorrhea and dental caries were not included, no matter how profound.

TABLE III

RESULTS OF TONSILLECTOMY ACCORDING TO AGE

AGE	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
Forty years of age or under	58	39	67.2
Over 40 years of age	45	21	46.7
Total	103	60	58.3

TABLE IV

RESULTS OF TONSILLECTOMY ACCORDING TO DURATION OF DISEASE

DURATION	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
One year or less	49	29	59.2
More than one year	54	31	57.4
Total	103	60	58.3

Altogether, 35 patients were studied and of these, 24, or 68.6 per cent, were improved (Table V). Five of the patients received no other therapy, while 30 were given in addition streptococcus vaccines of types similar to those used in conjunction with tonsillectomy. In the results of vaccine therapy alone, reported later in this paper, the percentage of improvement was low, and it seems reasonable, therefore, to attribute the high percentage of improvement chiefly to the removal of the dental foci. The average number of injections for patients in this group was 19.1.

TABLE V

RESULTS OF ABSCESSED TEETH EXTRACTION

	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
Teeth only	5	5	
Teeth and streptococcus vaccine	30	19	63.3
Total	35	24	68.6

The number of patients having teeth extraction is too small to warrant a detailed analytical study. They were somewhat older than those subjected to tonsillectomy, 11 being forty years of age or under, while 24 were over forty. The milder cases showed a greater tendency toward improvement than did the more severe.

OTHER FOCI OF INFECTION

Next to diseased tonsils and teeth, infected sinuses appeared most prominently as a focus of infection in patients with rheumatoid arthritis. Because the treatment of infected sinuses is generally so unsatisfactory, it was considered advisable to omit this form of therapy from the present study. In the Cornell Clinic, other foci of infection were seldom noted.

RESULTS OF VACCINE THERAPY ALONE

Altogether 194 patients were treated with vaccine therapy only, and of these, 69, or 35.6 per cent, showed improvement (Table VI). Of the 87 patients receiving "typical strain" vaccine subcutaneously 32, or 36.8 per cent, showed improvement; of the 36 receiving the same vaccine intravenously 13, or 36.1 per cent, showed improvement; while of the 48 receiving it both subcutaneously and intravenously 14, or 29.2 per cent, showed improvement. Twenty-three others were given various kinds of streptococcus vaccines with improvement in 10, or 43.5 per cent.

The average number of injections for patients in each group is indicated in Table VI.

Regardless of the type of vaccine or the method of administration, remarkably similar results were recorded.

TABLE VI

RESULTS OF STREPTOCOCCUS VACCINE

	NUMBER OF CASES	AVERAGE NUMBER INJECTIONS	NUMBER IMPROVED	PER CENT IMPROVED
"Typical Strain" vaccine, subcutaneous only	87	34.9	32	36.8
"Typical Strain" vaccine, intravenous only	36	15.7	13	36.1
"Typical Strain" vaccine, subcutaneous and intravenous	48	24.1	14	29.2
Other forms of streptococcus vaccine (subcutaneous only)	23	18.1	10	43.5
Total	194	26.7	69	35.6

TABLE VII

RESULTS OF STREPTOCOCCUS VACCINE ACCORDING TO SEVERITY OF DISEASE

	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
+	67	22	32.8
++	94	38	40.4
+++	33	9	27.3
Total	194	69	35.6

In Table VII, the results of treatment with vaccine only are tabulated according to the severity of the disease. The mild cases showed an improvement of 32.8 per cent, the well-developed, 40.4 per cent, and the severe, 27.3 per cent, results from which little can be deducted.

In Table VIII the patients treated with streptococcus vaccine only are grouped according to age. Those forty years of age or under showed an improvement of 44.3 per cent, while those over forty showed only 29.6 per cent improvement.

Table IX reports the results in the same group of patients as in Table VIII, but viewed from the standpoint of the duration of the disease. Of 77 patients having the disease for one year or less, 46.8 per cent improved, whereas, of 117 having the disease for more than one year, only 28.2 per cent were improved.

The results shown in Tables VIII and IX are extremely difficult to interpret. The figures may indicate that young patients and those having the disease for only a short time received some benefit from streptococcus vaccine therapy, on the other hand, they may only indicate that such patients have a somewhat greater tendency toward spontaneous self-limitation of the disease. To us the latter interpretation seems more probable.

TABLE VIII

RESULTS OF STREPTOCOCCUS VACCINE ACCORDING TO AGE

	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
Forty years of age or under	79	35	44.3
Over 40 years of age	115	34	29.6

TABLE IX

RESULTS OF STREPTOCOCCUS VACCINE ACCORDING TO DURATION OF DISEASE

	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
One year or less	77	36	46.8
More than one year	117	33	28.2

SUMMARY AND DISCUSSION

This paper is a presentation of the results of the treatment of 332 patients with rheumatoid arthritis by removal of foci of infection and with the administration of certain streptococcus vaccines.

One hundred and three patients were treated by removing diseased tonsils and 60 (58.3 per cent) showed subsequent improvement of their arthritic condition. Twenty of these had no other form of therapy, while 83 were given in addition streptococcus vaccine. No higher rate of improvement was noted in patients having vaccine and tonsillectomy combined than in those having tonsillectomy alone.

As would be expected, the results in the tonsillectomy group were best in young patients and those having mild arthritis; the duration of the disease appeared to be an unimportant factor in the chances of recovery.

Thirty-five patients had abscessed teeth removed, of which 24 (68.6 per cent) showed improvement. Thirty of the thirty-five received streptococcus

vaccine therapy also. Taking into consideration the low percentage of improvement in patients having vaccine therapy only, it seems reasonable to assume that the high percentage of improvement in patients with dental extraction and vaccine therapy can be largely attributed to the removal of the abscessed teeth per se.

One hundred and ninety-four patients were treated with vaccine therapy alone. Of these only 69 (35.6 per cent) showed improvement. The results were remarkably similar, regardless of the type of vaccine used, or of the method of administration.

Throughout this study, complete and permanent cure was an exceedingly rare occurrence. The removal of diseased tonsils and abscessed teeth, however, favorably modified the course of the disease in a high percentage of cases.

Although our policy has been to advise removal of foci only when there was strong evidence that they were a source of infection, we feel that perhaps this strict ruling has in some instances permitted the retention of foci responsible for the arthritic condition.

In estimating the results of vaccine therapy in rheumatoid arthritis, one is impressed with the fact that we have no measuring rod for accurately determining the severity of the disease in any individual patient. The erythrocytic sedimentation test offers possibilities for such a determination, but further work must be done in this field.

That vaccine inoculation undoubtedly has a considerable psychologic effect on the patient should be taken into consideration in estimating the results of this type of therapy.

In regard to the series of cases here reported, it is, of course, admitted that the employment of other forms of streptococci or other methods of preparing vaccine might have been attended with more satisfactory results.

Although we do not know the percentage of patients who would have improved spontaneously, our results with vaccine therapy were extremely disappointing, as the low percentage of improvement may well represent the natural tendency of some patients to improve regardless of treatment, rather than to any inherent value in streptococcus vaccine therapy.

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YEAST-LIKE FUNGI*

Differential Characteristics and Case Reports

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THE literature shows a wide disagreement among investigators as to the cultural, biochemical, and morphologic characteristics of yeast-like fungi parasitic to man. Robins[1] first studied the morphologic characteristics of the thrush fungus and, not recognizing the similarity of this fungus to M. candida described previously by Bonorden,[2] he named it oidium albicans, oidium being the term applied by Link, 1809, to the fungus of grapevine mildew and later by Fresenius, 1850, to name a fungus isolated from sour milk. The name of the thrush fungus has changed several times since its recognition. Reess,[3] 1870, reported the presence of asci and called it saccharomyces albicans. After the advent of pure cultural methods, Zopf[4] found no asci in the thrush fungus, recognized its identity with yeast-like fungi of the genus monilia and renamed the organism Monilia albicans. The nomenclature was again changed by Vuillemin,[5] 1898, when he reported the formation of asci. He named it Endomyces albicans. Since then the work of Zopf has been substantiated and the organism has retained the name given by him, M. albicans.

Multiplicity of species among the cultures isolated from cases of thrush was recognized by Plaut,[6] 1887. He studied cultures of what he designated as "Thrush Fungus" and M. candida and came to the conclusion that from the standpoint of morphology and pathogenicity they are identical. Multiplicity was also recognized by Herff,[7] 1895. He observed that organisms of this group were associated with disease processes involving the mucosa of the vagina. He described 26 cases of vaginitis due to a yeast-like fungus, and identified the organism from 4 of the cases as M. candida and from 16 as M. albicans. Craik,[8] 1913, also recognized the multiplicity of species in this genus and described both M. candida and M. albicans.

The term "blastomyces" was first used by Busse[9] in Germany and Gilchrist in this country to designate a yeast-like pathogenic fungus the exact

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identity of which was puzzling. Since then the term blastomycosis has come to cover all disease processes with which a yeast-like organism is associated, and the term blastomyces to designate such organisms as saccharomyces, cryptococci, monilia, oidium, torula, and endomyces.

Recently there has been an effort to classify these organisms more strictly. For this purpose sugar fermentations, litmus milk, and gelatin reactions have been used. The variable results from these studies have caused considerable confusion and the consequence has been a great increase in the multiplicity of species especially in the monilia group. Castellani identified up to 1920 forty-two different species of monilia. Later, disregarding acid and using only gas production in certain sugars, together with gelatin and litmus milk reactions, he reduced this number to 28.

Following this work the literature abounds with reports of yeast-like fungi associated with various disease conditions, bronchitis, pneumonia, asthma, thrush, vaginitis, etc. The organisms reported are given a variety of names. From this review, it is obvious that the literature is confusing as to the classification of the yeast-like fungi pathogenic for man and particularly as it applies to monilia. All of the investigators have reported a variability of these organisms in their cultural and biochemical characteristics. This variability has been accepted as due to the variability of the organisms under identical environmental circumstances.

Because of this confusion and because we saw frequently in sputum, which was being examined for tubercle bacilli, yeast-like cells and mycelium, we became interested in their identification and classification. In 1928,[10] one of us, Stovall, reported 18 cases of primary pulmonary infection with yeast-like fungi. No other name was given to the organisms isolated, because we were confused as to their identity. Since then we have isolated 200 cultures from cases of bronchitis, pneumonia, asthma, bronchiectasis, thrush, and vaginitis. In 1929[11] we reported a cultural study of 40 of these strains. We did not encounter the variability that has been so frequently reported, but to the contrary when it was possible to repeatedly establish the same environment as to composition of media, temperature, time of incubation and to observe the results at the same time in the incubation period, we found that the individual organisms and those in the same species showed remarkably constant morphologic, cultural and biochemical characteristics. Among these cultures we encountered only three species of monilia.

We were impressed with the constancy of the results and also with the limited number of species encountered. We thought that possibly the latter experience was due to the locality and therefore decided to study cultures of monilia secured from the American type culture collection. We selected certain cultures because of the investigators who first studied them and also because of the locality in which the investigator was working at the time the organism was isolated. In this study[12] we found that all of the cultures of monilia received from the American type culture collection could be classified with one of the species we had identified among our cultures, and also that when submitted to our method of study, they were as constant in their behavior to the various medias as our own cultures.

We have now completed the study of 250 cultures isolated by one of us (Bubolz) from cases of bronchitis, pneumonia, asthma, thrush, vaginitis, and generalized infections. We find that all of the monilias behave in a constant manner and that among all of these cultures of monilia there exist only three species.

This paper is for the purpose of a detailed description of the methods we have employed and also to record the different varieties of organisms which we have encountered together with the clinical characteristics associated with these infections.

MATERIAL AND METHODS

The 250 organisms used in the study were isolated from patients suffering from bronchitis, asthma, pneumonia, thrush and vaginitis, and cultures obtained from the American type culture collection.

Microscopic.—The sputum was examined first by making moist preparation for microscopic study. The material was selected for these preparations

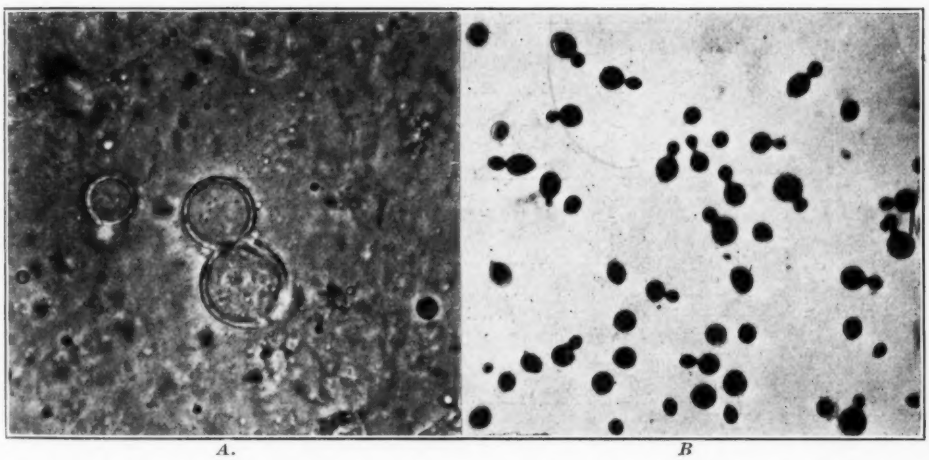
Fig. 1.—Monilia in sputum.

by pouring the sputum into a sterile Petri dish and, with the black of the table top for a background, picking the small flakes of grayish white material. These small flakes are composed of tangled threads of mycelium, epithelial cells and fibrin. They are loosely held together and as a rule no sodium hydroxide is needed to soften them. In some instances when the material has been too tough and hard to satisfactorily press out under a coverslip, we have treated it with a 20 per cent sodium hydroxide solution. This method of examination reveals in diseases associated with the presence of certain yeast-like fungi, monilia, and endomyces, many tangled threads of mycelium and many yeast-like cells some of which are seen to bud (Fig. 1). In other varieties of yeast-like fungi, oidia and torula, no mycelium is seen but the yeast-like cells are present (Fig. 2, *A* and *B*). Occasionally this examination will fail to reveal the organisms which will show up in the cultures. It is important, however, to demonstrate the organism in the moist preparation since it lends considerable emphasis to the interpretation of the finding. Their presence in large numbers in the sputum, particularly in the

grayish-white particles, certainly associates them with the exudate and secretion coughed up from the bronchi and rules out mouth contamination. This is particularly true if no mouth lesions can be demonstrated. The interpretation of positive cultural results and negative moist preparations requires repeated study in the laboratory as well as at the bedside.

In the case of thrush or vaginitis, swabs are used to collect the exudate, and from these glass slides, smears and cultures are made. The smears are stained by Gram's method and reveal either yeast-like cells or mycelium or both.

Isolation.—This can be accomplished from 1 per cent glucose broth cultures or directly from sputum or inflammatory exudates. The culture or exudate is plated on malt and plain agar. The plates are incubated at 37° C. In our experience any of these fungi except oidium will show on malt agar, after forty-eight hours incubation, colonies measuring from 2 to 5 cm. Oidium



A. *B*

Fig. 2.—*A*, Oidia (blastomycetes) in sputum. *B*, Torula; forty-eight hour broth culture.

does not develop so rapidly on malt agar but develops similar sized colonies on plain agar in a week to ten days.

Pure Culture Study.—This is made for the further identification and classification of the organisms. For this purpose we have studied morphology, method of reproduction, colony morphology on malt agar plates, fermentation of various carbohydrates, gelatin liquefaction, and reactions in milk.

On malt agar plates the monilias show characteristic colony morphology for the three species at the end of forty-eight hours incubation at 37° C. (Fig. 3, *A*, *B*, and *C*.) The various genera, endomyces, torula, oidia, are differentiated from the monilia group and from each other by morphologic studies and their method of reproduction (asci formation), simple budding, mycelium, and the formation of asci.

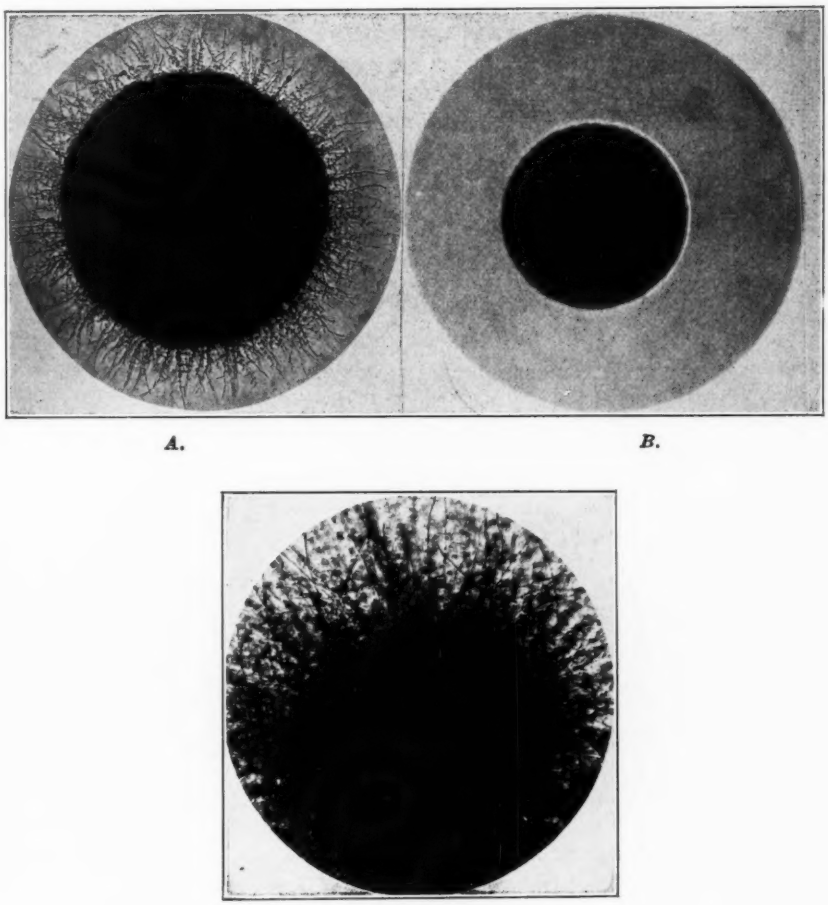
Fermentation.—For purposes of specie identification of monilia, we found the sugars indicated in Table II to give distinguishing reactions. These sugars are added to plain Liebig's extract broth in sufficient amounts to

TABLE 1*

TYPE OF MONILIA	NUMBER AND NAME OF CULTURE	DEXTROSE	LEVULOSE	MANNOSE	MALTOSE	GALACTOSE	SACCHAROSE	LACTOSE	INULIN	RAFFINOSE	DEXTRIN	MYCELIAL COLONIES ON MALT AGAR IN 48 HOURS	10% GELATIN	MILK
I	Monilia Type I	AG	AG	AG	A	A	A	0	0	0	0	+	0	0
II	Monilia Type II	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	2112 M. albicans-Craik	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	801 M. richmondi Shaw	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	2117 M. psilosis ashfordi	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	752 M. pinoyl castellani	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	753 M. metalondinensis castellani	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	4021 Monilia-Zillig	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	4135 M. pseudotropicalis castellani	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
III	Monilia, Type III	AG	AG	AG	AG	AG	AG	0	0	0	0	++	0	0
	2113 M. candida bonorden	AG	AG	AG	AG	AG	AG	0	0	0	0	++	0	0
	1369 M. candida bonorden	AG	AG	AG	AG	AG	AG	0	0	0	0	++	0	0
	750 M. tropicalis castellani	AG	AG	AG	AG	AG	AG	0	0	0	0	++	0	0
(Endomyces)	748 M. macedoniensis castellani	AG	AG	AG	0	AG	AG	0	AG	AG	0	+	0	0
(Mycoderma?)	749 M. krusei castellani	AGP	AGP	AGP	0	0	0	0	0	0	0	+	0	0

*A = acid; G = gas; O = negative results; + = postive; C = clot; and P = pedicle.

make the broth a 1 per cent solution and the final P_H adjusted to 7.2. Brom thymol blue indicator is added to the broth. The broth is inoculated from a forty-eight hour agar slope culture and incubated for seven days. The cultures are observed at the end of forty-eight hours and four days but a final reading is not taken until the end of the seventh day. They are observed for the formation of acid and gas.



A. *B.*

C.

Fig. 3.—*A*, Monilia, Type I; forty-eight hour malt agar colony. *B*, Monilia, Type II; forty-eight hour malt agar colony. *C*, Monilia, Type III; colony of M. candida.

Gelatin.—We have found no monilia which will liquefy 10 per cent standard gelatin and therefore we do not use it for the routine identification of species in this group.

Milk.—We prepare a special milk media to insure constancy of results. Skimmed milk is tubed in 10 c.c. quantities and "Arnolded" on three successive days after which 0.5 c.c. of a sterile 10 per cent calcium lactate solution is added to each tube. This media is inoculated with 0.1 c.c. of a salt solution suspension made by washing a forty-eight hour growth from a malt agar slope, and observed for coagulation for a period of one week. The

incubation temperature is 37° C. and the cultures are inspected every twenty-four hours during the test.

CLINICAL OBSERVATION

Monilia.—The genus identification is made on the basis of morphology and method of reproduction. The species in the genus are determined by a study of the cultural behavior toward certain sugar media, calcium lactate milk, and colony morphology on malt agar after forty-eight hours incubation at 37° C.

Morphology.—All of the cultures, 250, studied reproduce by budding and mycelial formation. The spores vary greatly in size and morphology. In the same culture small and large cells are seen as well as round and oval forms. The mycelium is septate and lateral conidia form by budding near the joints of the hyphae and terminal conidia by budding and constriction at

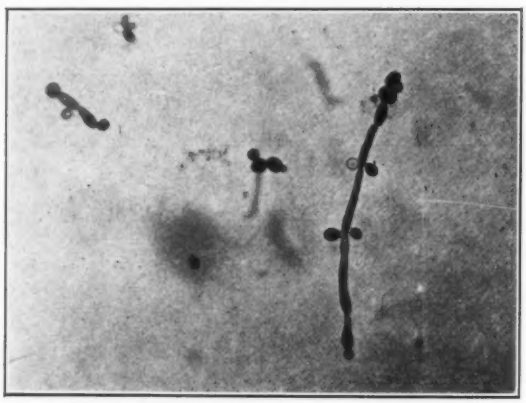


Fig. 4.—Monilia; lateral and terminal conidia.

the ends of the hyphae (Fig. 4). We have found no morphologic variation in the genus upon which to base a species differentiation. Asci have not been demonstrated.

The cultural and biochemical reactions which we have found to be characteristic for specie differentiation are shown in Table II.

The relation of monilia to disease has been disputed. There are, however, many clinical reports, Castellani, Ashford, Sen, Paramanad, Piper, Boggs, Pincoff, Simon, Steinfied, Johns and Shaw, and others have associated monilias as etiologic factors with bronchitis, asthma, pneumonia, thrush, and vaginitis. In all of these reports either no attempt has been made to classify

TABLE II

SPECIES	MALTOSE	SACCHAROSE	MILK	MYCELIAL COLONIES
Type I	Acid	Acid	0	+
Type II (M. albicans)	Acid and gas	Acid	Coagulation	0
Type III (M. candida)	Acid and gas	Acid and gas	0	++

the fungus isolated, or Castellani's classification has been adopted. We have isolated and classified monilia from lesions in various locations in the body and have found the types described above. We have also found that in one patient vaginitis may be caused by M. candida and in another by M. albicans. This has also been true for cases of thrush, bronchitis, and asthma. While we have found Group I monilia in sputum, we have never found it to be associated with thrush or vaginitis. The brief case histories below are illustrative of our clinical experience with the monilias.

CASE 1.—White male, aged fifty-two years. Came on Jan. 15, 1925, complaining of a sore mouth. He said that he had been suffering from this condition for two years and that one year previous he had been told it was due to a streptococcus infection due to bad teeth. He had his teeth removed at that time. The examination revealed a membranous exudate covering the mucous membrane of the cheeks, tongue and lips. The membrane was thin and was removed in small pieces. Direct smears stained with Gram's stain revealed a yeast-like fungus with abundant mycelium. Cultural identity, M. candida.

CASE 2.—J. C., white male, aged five years, admitted to the hospital Feb. 4, 1931, weight 39 pounds. The parents said the child had been drooling since five months of age and had had a photophobia with yellow discharge from the eyes since one year of age. They had also noticed white patches in the mouth since he was a year old and about three years ago he had a severe infection of the mouth accompanied by acute otitis media.

The examination revealed a pale, anemic child much underweight. The corneal reflex was lost. There were a few corneal opacities in the upper half of the left cornea. In the mouth there were found many white elevated patches covering the mucous membrane and tongue. These lesions were typical of thrush. Over the palmar surfaces of the hands there was a scaling and pealing and the finger nails were thickened and discolored. These lesions were typical of epidermophyton infection. Smears made from the lesions in the mouth showed a yeast-like fungus with abundant mycelium. The cultural identity of this fungus was M. albicans. It was also isolated from the stool. Epidermophyton microsporon was isolated from the finger nails.

CASE 3.—R. K., a man, aged thirty-six, a laborer, complained of coughing up blood. There was no family history of tuberculosis. Fourteen weeks before his admission he had an upper respiratory infection and had developed a cough. About a week later he had a chill following exposure. Following the chill he gradually became more short of breath and began to cough up bright red blood. He continued to cough up blood and felt weak. Three weeks later he consulted a physician who took an x-ray of his chest and told him he had tuberculosis.

Direct examination of sputum did not reveal a yeast-like fungus but the cultures grew a yeast-like fungus which was identified as Monilia Type I. Sputum examinations for tubercle bacilli were repeatedly negative.

CASE 4.—E. S., aged fifteen, a schoolboy, admitted to the hospital Jan. 16, 1928, had developed a dry, nonproductive cough beginning about a year before. In September, 1927, he had begun to have afternoon fever which rose to about 100° F. He did not raise any sputum at this time although he still had a cough. December 12 he went to bed because of weakness. A diagnosis of bronchitis was made by the local physician. Jan. 4, 1928, the patient returned to school. He felt exhausted but there was still no sputum. January 14 he developed a high fever and for the first time raised about a teaspoonful of blood stained sputum. The patient had lost ten pounds in the past six months. The sputum over a period of a year and a half was repeatedly positive for M. albicans and negative for tubercle bacilli.

CASE 5.—A. W., a man, aged forty-five, was admitted to the hospital Feb. 20, 1928, in a moribund condition. His local physician said that early in December he had influenza.

He was ill about four weeks at that time. He continued to cough a great deal but the sputum was not blood stained until the latter part of January. He had complained of sore throat and his voice was husky. The throat condition grew worse rapidly and his cough was almost continuous when he was admitted to the hospital. At this time he had a large ulcer involving the soft palate and another on the upper lip. Two weeks prior to his admission

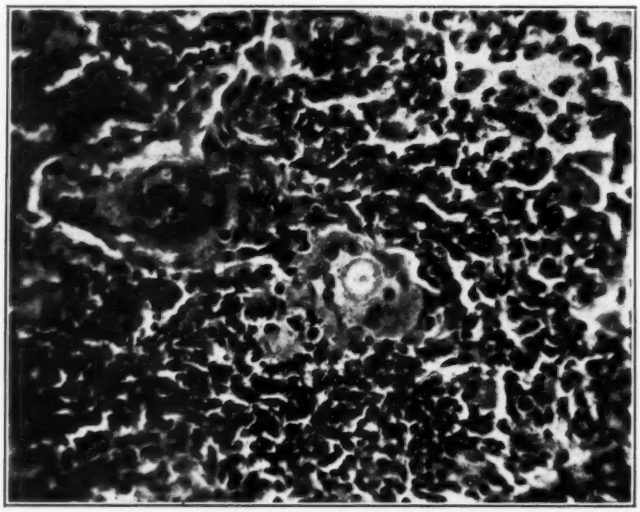


Fig. 5.—Blastomyces spore in a giant cell.

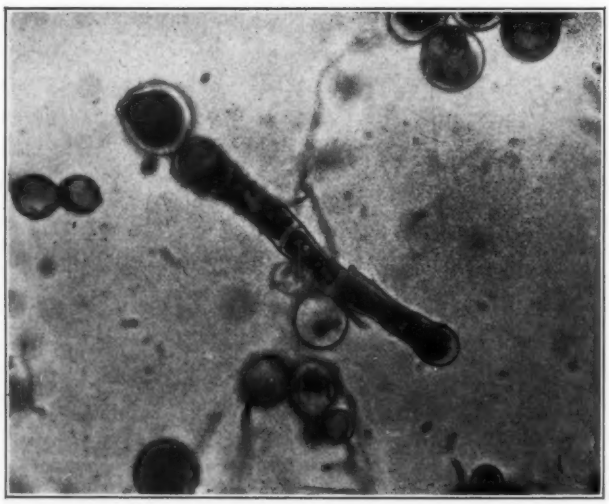


Fig. 6.—Blastomycetes (Oidia); septation and abstriction of hyphae.

to the hospital a direct examination of the sputum revealed large numbers of yeast-like cells and mycelial threads in the sputum. The cultural results identified M. albicans. Examination of secretion from the ulcers revealed the same organisms.

Oidium.—(Blastomyces) We have isolated two cultures and with these have studied one, Asteroides, Castellani, secured from the American type cul-

ture collection. The fermentation reaction, colony morphology and other cultural characteristics are described in Table III. This organism we believe to be the same as the one described by Busse and Gilchrist as the cause of blastomycosis. In tissue this organism is seen as single, large spores, 8 to 10 microns in diameter, some of which can be seen to be budding. They are often seen in the giant cells which are part of the tissue reaction (Fig. 5). Cultured on solid media it soon produces profuse mycellium, the colony finally being covered by a downy, fuzzy growth. Spores are formed from the mycelium by abstriction and septation of the hyphae (Fig. 6). This organism is easily distinguished from the other yeast-like fungi described in this paper by the size of the spores and their double contoured wall, and by the very coarse mycelium. The fermentation further differentiates this organism. It ferments no sugar even after long periods of incubation, thirty days.

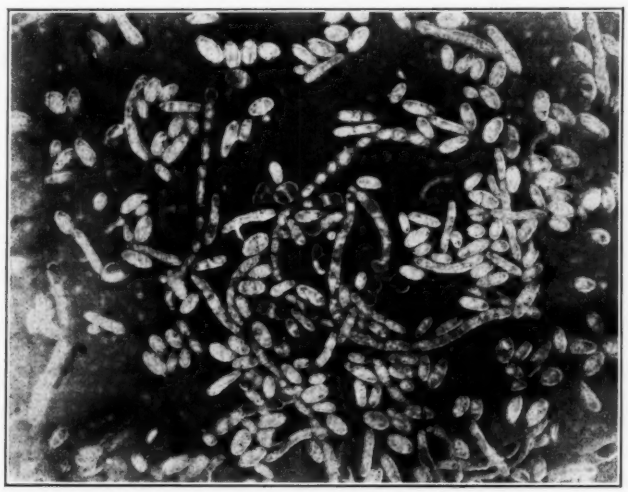


Fig. 7.—Asci in culture of endomyces.

Case 1.—C. G., aged seventeen years; admitted to the hospital on Sept. 3, 1929, complaining of pain in the chest. He had been ill since May, 1928. He had been in bed since September, 1928. He had impairment of function of joints of the hands, knees, elbows and shoulders since August, 1929. The physical examination revealed a poorly nourished and anemic male; marked lag in respiration in the left chest; impaired resonance in both apices, more marked on the left; tactile and vocal fremitus decreased over the same area; high pitched inspiration and prolonged expiration; systolic murmur over the aortic area transmitted to the vessels of the neck; liver palpable one and one-half centimeters below the costal margin; edema of the lower extremities; fluid in both knee joints; hypertrophic joint changes in the knees, feet, and hands. Probable diagnosis at this point, pulmonary tuberculosis, chronic infectious arthritis, secondary anemia, chronic pharyngitis, and dental sepsis.

The sputum examination was negative for tubercle bacilli after many specimens had been examined. The direct examination of the sputum showed large double contoured spores and no mycelium. The cultural identity of the organism was oidium (blastomyces). Clinical diagnosis, blastomycosis.

Endomyces.—This organism reproduces by budding, and mycelial and asci formation. The mycelium is septate and conidia are formed laterally and at

the joints of the hyphae, and by abstriction at the ends of the hyphae. There are minor morphologic differences between this organism and the monilias. The chief distinguishing characteristics are the production of asci (Fig. 7) and the sugar fermentation.

It has been our experience that a yeast-like fungus giving the fermentations recorded for this organism in Table III has always shown asci and has turned out to be endomyces. We have now isolated this organism from three specimens of sputum. The study of three cultures is, of course, a limited experience. However, the fermentation of lactose, inulin, and raffinose

TABLE III

	GLUCOSE	LEVULOSE	MANNOSE	MALTOSE	GALACTOSE	SUCROSE	LACTOSE	INULIN	RAFFINOSE	DEXTRIN	GELATIN	MILK	MYCELIAL COLONIES MALT AGAR 48 HOURS
Monilia Type I	AG	AG	AG	A	A	A	0	0	0	0	0	0	+
Monilia Type II	AG	AG	AG	AG	AG	A	0	0	0	0	0	C	0
Monilia Type III	AG	AG	AG	AG	AG	AG	0	0	0	0	0	0	++
Endomyces	AG	AG	AG	0	AG	AG	AG	AG	AG	0	+	A	+
Saccharomyces	AG	AG	AG	AG	AG	AG	0	0	AG	0	0	0	0
													+
Oidia	0	0	0	0	0	0	0	0	0	0	+	0	12 days
Torula	A	A	A	0	A	A	0	0	0	0		0	0

has been a constant characteristic of these three strains. The organism secured from the American type culture collection, classified as M. macedoniensis, produced asci in our study and we therefore recognized it as an endomycete. It did not, however, ferment lactose. It appears then that a study of a large number of strains might reveal a variety of species.

CASE A. S.—This patient, a woman thirty-five years of age, was admitted to the hospital on Dec. 11, 1927, with a severe rhinopharyngitis and bronchitis. After two or three days in bed she appeared to be improved but she continued to have a low fever and a few râles at the base of both lungs. This condition persisted for two more days when an area of consolidation was found in the right lung posteriorly. The patient's fever continued about the same, 99.6°-101°, but the chest manifestations did not improve.

X-ray plates made December 29 showed enlargement of hilum lymph nodes on the right side, increased visualization of peribronchial structures, several organized spots scattered through the lower half of the right lung, and a fogging in the left apex.

Sputum was sent to the laboratory December 23 with a request for an examination for acid fast bacilli. This was repeated for five successive days and each time reported negative. However, on December 26 the laboratory reported the presence of a yeast-like fungus. The organism proved to be an endomycete.

The patient recovered and was discharged from the hospital on December 20. She has been followed closely and at the present time x-ray plates of her chest are clear and she is in good health.

Torula.—We have studied six cultures, four isolated by us and two from the American type culture collection. The morphology of this organism is like that of saccharomyces except that no asci have been demonstrated. It reproduces chiefly by budding. However, in broth cultures left at room temperature for three months, abundant mycelium is produced in the sugars in which acid is produced. No gas is formed in sugars, acid being produced slowly in glucose, levulose, mannose, galactose, and saccharose. We have encountered two varieties of colonies, one a creamy, glossy colony, and the other a salmon to pink color. The colonies are more glossy than the monilias and saccharomyces.

CASE J. L.—This was a white male patient, twenty-three years of age. He has suffered from asthma ever since he was two and one-half years old. His asthma followed an attack of pneumonia. His condition had not increased in severity. He sometimes had quite severe attacks of dyspnea. He said he had noticed a sensitiveness to various dusts and to the eminations from cats and dogs.

The physical examination revealed a congested nasal and pharyngeal mucosa and emphysematous lungs.

The laboratory examinations were essentially negative, except for the sputum examination. The x-ray showed nothing important. The sputum cultures revealed a yeast-like fungus, which was identified as torula (white).

SUMMARY

This paper briefly reviews the literature on the classification of yeast-like fungi and points out the confusion which has led to a multiplicity of species for monilias and resulted in the classification of all yeast-like fungi as blastomyces.

The methods used and the characteristic differences of certain of these types of fungi are described. The differences are found to be characteristic for the various species and when the organisms are observed under the conditions described, they are constant in their behavior, showing no variation after artificial cultivation over a period of many years.

This study is associated with a brief report of cases infected with different kinds of yeast-like fungi. These cases indicate the clinical manifestations of these infections. This report suggests that these organisms may be the cause of bronchial asthma in certain cases. The cases also demonstrate that these organisms may be associated with lesions in various anatomic locations; that is, M. candida may in one patient be associated with thrush and in another with vaginitis.

This study further indicates that yeast-like fungi can by careful clinical and laboratory studies be established as the primary etiologic agent in certain cases of pulmonary, vaginal and mouth mucous membrane inflammatory processes. On the other hand such cases as J. L. (torula) raise the question as to the significance of this organism in an allergic patient.

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ON THE ADVANTAGE OF ALTERNATING THE VEGETABLE AND METALLIC DIURETICS IN THE TREATMENT OF EDEMA OF CONGESTIVE HEART FAILURE*

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GALVESTON, TEXAS

IN THE prosecution of our studies[1-5] on the mechanism of the action of various types of diuretics, we were impressed with the influence that the order or sequence in which the drugs were administered had upon the type and degree of diuresis. In our second (repeated) compound or triple drug experiment (William Blake), our results as to the filtration and reabsorption seemed to refute our original findings and conceptions, but the idea of a possibility of "hang over" or persistence of effects was immediately conceived and steps were taken to verify such an explanation or contention. (Tables I, II, and III, Figs. 1, 2, 3, and 4.)

This evidence we felt would in turn help to substantiate the conception of the purine's increased glomerular filtration and the heavy metal's decreased tubular reabsorption mechanisms of diuresis with which we were concerned. These observations and facts suggested a possible complementary therapeutic combination of the two modes of action by a proper sequential or simultaneous administration of the two types of diuretics.

It was evident that augmentation of diuresis under such would have a distinctly practical value and should be put to extensive clinical investigation.

This has been done in the medical wards of the John Sealy Hospital and the validity of the arguments seems to us to be established almost beyond peradventure. We can administer combinations which almost uniformly

*From the Dr. Edward Randall Laboratory of Clinical Research of the Department of Medicine of the University of Texas Medical School and the John Sealy Hospital. Diuresis Study VI.

yielded better results than did the two drugs previously administered alone. We have also been successful in instances where one drug alone in the same dosage had failed. In general it furthermore has been possible to accomplish diuresis with smaller doses of the drugs, doses that for either drug alone would be ineffectual. The great enhancement of the urinary output brought about as a result of purposeful premeditated arrangement of the time and order of drug administration, by virtue of fact that the drugs in our way of thinking act on different parts of the renal unit, struck us as a distinctly practical therapeutic procedure.

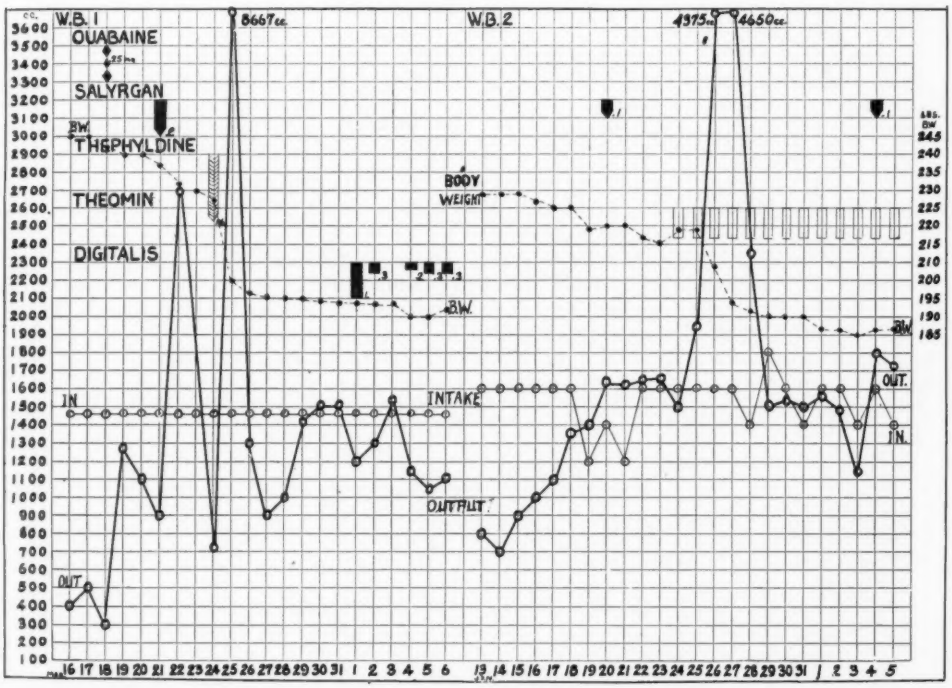

Fig. 1.—Clinical observation of the daily body weight, fluid intake and urine output over a period of twenty-two days of the first and twenty-four days of the second admission of W. B., colored, aged forty-three, with syphilitic aortic regurgitation in extreme congestive failure with anasarca. Complete studies were made by the Rehberg creatinine method of the mechanism of the diuresis following the intravenous injection first of ouabaine, second three days later of salyrgan, and three days after this the third acute experiment was done with theophylline ethylene diamine and finally digitalization was carried out. The results of the acute experiments with regard to (*F*) filtration and (*R*) reabsorption will be shown in Figs. 2, 3, and 4. Note the great augmentation of the output that resulted from the theophylline that followed the mercurial. The second admission shows the efficiency of theophylline by mouth following the mercurial by vein.

Just as Schmitz[7, 8] using the Rehberg[6] creatinine method in the study of the mechanism of experimental diuresis in animals has shown, so we too have demonstrated in patients with congestive heart failure and edema that the metallic diuretics, especially the mercurials, accomplish their results by adversely affecting the permeability of the tubular epithelium and decreasing the tubular reabsorption primarily, while the purine derivatives, especially theophylline and in our experience even digitalis bodies, produce diuresis principally by increasing the glomerular filtration. The opening up of more renal

units by the vascular effects of the xanthines probably also plays a minor part as does the increased rate of flow through the tubular capillary plexus in the increased efficiency of urine excretion.

Theophylline ethylene diamine (thephyldine), theophylline, the drug in the purine or xanthine series that we have used most often has definite augmentory central circulatory, as well as renal effects. It is primarily used as a vasodilator and is generally considered to have its effects on the active arteriolar vascular sector in the heart and kidney, but probably also in the other peripheral parts of the vascular beds. Mercury salicyl-allylamide-o-acetate of sodium, mersalyl (salyrgan), the mercurial used is apparently fil-

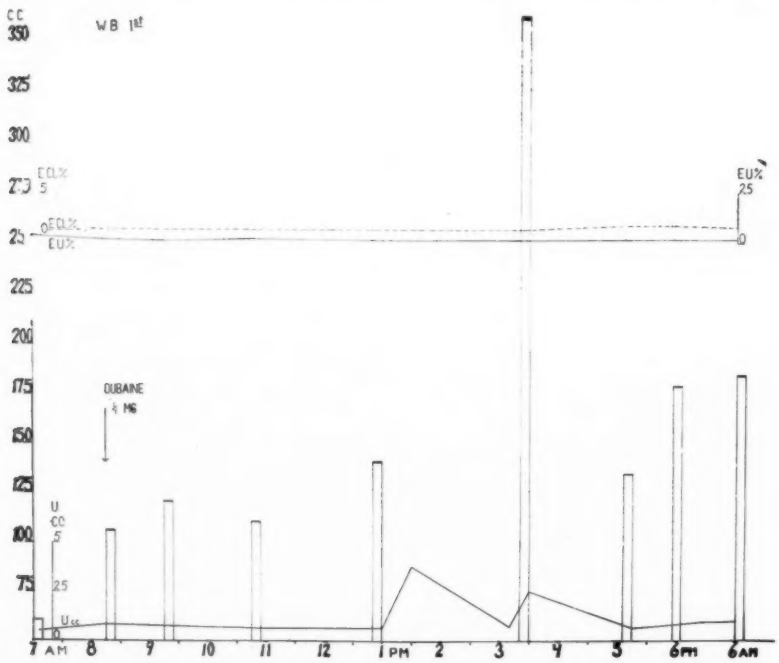

Fig. 2.—A schematic representation of the results obtained by the creatinine method of estimation glomerular filtration (F) and tubular reabsorption (R) following the intravenous injection of ouabaine. The total heights of the columns to the very top represents the filtration rate (F) per minute in cubic centimeters; the columns to the lower margins of the black cap represent the reabsorption (R) rate per minute in cubic centimeters; the black cap itself, the difference between F and R, is the urinary output rate in cubic centimeters per minute (U). The actual urinary excretion per minute on a scale 20 times that used for F, R and U is shown by the connected line at the bottom U c.c. obtained by dividing each urinary output in cubic centimeters by its interval in minutes and charting the same opposite the time at which each specimen was voided.

In the upper part of the graph there is charted the percentage of the filtered urea (EU per cent) and chlorides (ECl per cent) excreted at the time intervals throughout the day.

Note that after ouabaine just as was the case with digalen there was a striking increase in filtration and only a slight decrease in reabsorption slightly delayed, possibly because it depended on central or cardiac improvement in circulation and later improvement in glomerular circulation.

tered in dilute solution through the glomerular membrane, and as it comes in contact with the tubular epithelium and is diffused through in minute amount or reabsorbed, it affects the protoplasm of the cells of the tubular membrane and decreases their permeability. If the dose of mercury is too great, or the cells of the tubular epithelium are unusually susceptible, destruction results,

and there is an exaggerated or complete permeability with a resulting anuria. This has apparently happened in a few of our cases in which large 2 c.c. doses of salyrgan were given intravenously. Upon the basis of these facts of the modus operandi of each type of diuretic, the results of our experiment served to substantiate our contentions. We have studied these combination therapeutic effects in a series of 13 successfully and 2 unsuccessfully treated patients in congestive heart failure.

METHOD

All edematous patients admitted to the medical wards of the John Sealy Hospital are strictly confined to bed with a back rest and pillows as desired to make them comfortable. A low protein (40G) salt poor diet (2G) is prescribed with 200 c.c. of fluid at two or three

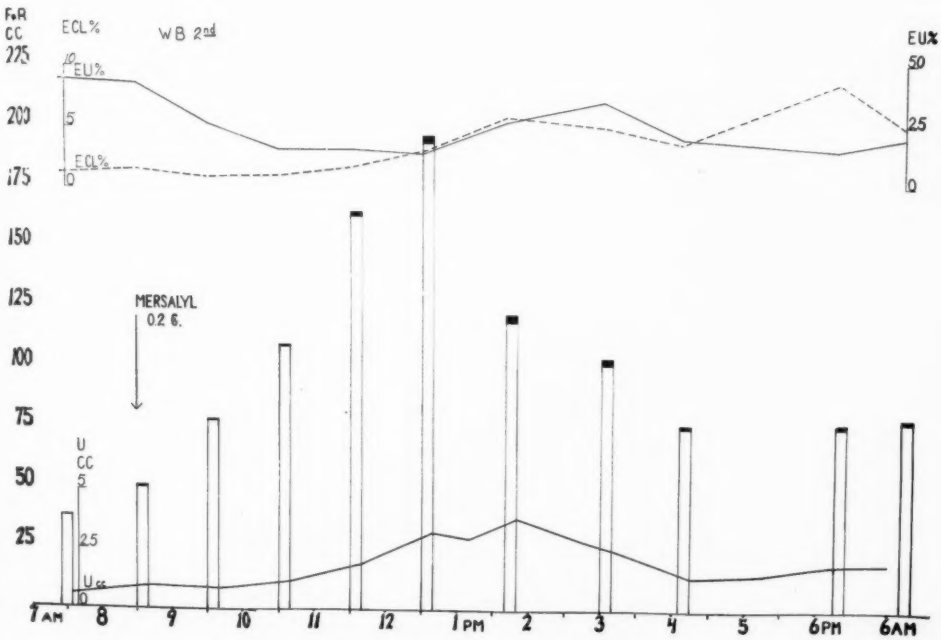

Fig. 3.—The description given for the symbols in Fig. 2 will suffice for this graph. In the experiment depicted here, the second one, the mercurial salyrgan was injected intravenously and the diuresis was accomplished, not in the pure salyrgan way by depressing tubular absorption (increase in black caps) but there was an increase in filtration which was considered as a persistence of the general circulation improvement effect of the ouabaine previously given.

hourly intervals for 8 or 5 administrations according to the degree of edema that the patient presents totaling 1600 c.c. or 1000 c.c. for the twenty-four-hour period. The degree of edema is roughly estimated as Grade 1 when there is just pitting; Grade 2 when it extends to the knees; Grade 3, to the scrotum; Grade 4, to the back with ascites; Grade 5, general anasarca. The body weight and height are recorded. All urine specimens are collected separately ending always a twelve-hour period by empting the bladder at 6 P.M. and 6 A.M. to complete the twelve- and twenty-four-hour outputs. Morphine sulphate is used ad lib. to quiet and keep the patient at ease.

On the first morning in the hospital before breakfast, a 30 c.c. blood sample is taken without stasis and under mineral oil for complete chemical analysis, two urine samples at

known time intervals are collected for volume rate per minute and urea clearance determination, and the complete twelve- and twenty-four-hour specimens are chemically analyzed.

The patient then is kept at rest in bed for three days on the same diet with the intake of fluid output of urine and the body weight recorded and charted daily.

If after three days, there is no rise in the output, the régime of experimental therapeutics is begun. If, however, there is any indication of a rising level of urinary output, the experiment is postponed for as many days as seems necessary to establish a baseline. On the first experimental day, the 30 c.c. of blood and the first two urine specimens and the twelve- and the twenty-four-hour specimens are again collected for analysis, and then the drug is given.

Theophylline ethylene diamine 0.48 gm. in 10 c.c. of physiologic salt solution is introduced intravenously and the time recorded. Each urine specimen voided, thereafter, is col-

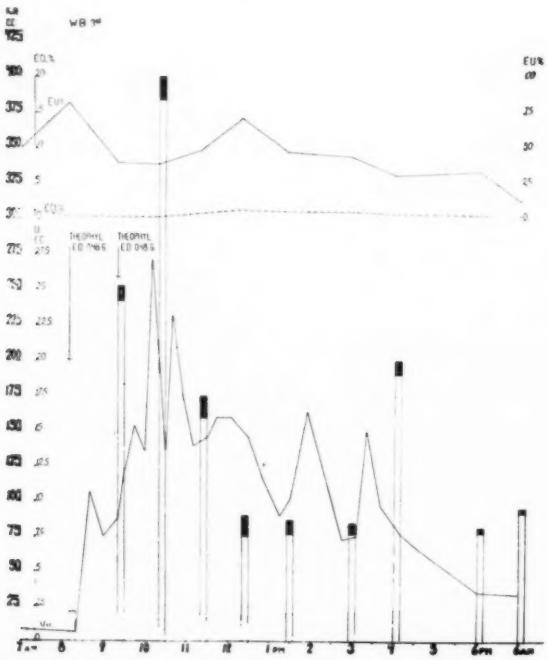

Fig. 4.—Again the description of the graph given under Fig. 2 is to be applied here. The third experiment, the result of injecting theophylline, shows a tremendous increase in the glomerular filtration per minute to a maximum of 400 c.c., with this the strikingly prominent black caps indicate a conspicuous defect in reabsorption, a persistence of the previous mercurial effect, with as a result a diuresis of almost nine liters of urine in twenty-four hours.

lected in a separate or individual bottle, the time is noted, the interval calculated in minutes, the urine volume and specific gravity are determined, and the rate per minute estimated. From this data can be determined; the time after injection of onset of diuresis or augmented or increased urinary flow, the time interval (after injection) elapsing before the maximum diuresis is obtained and the length of the diuretic period on this control dose of theophylline ethylene diamine (0.48 gm.) or of any other drug that may be chosen. The second experimental period is a similar one but for the change in the drug introduced intravenously.

As soon as the baseline of urinary excretion is reached and the glomerular filtration effect has disappeared, which is usually within a day except for digitalization effects that are more persistent, the second drug, the mercurial, is administered under the conditions of

the first experimental day. The preinjection blood and urine samples are taken for analysis; 1 c.c. of a 10 per cent solution (0.1 gm.) of salyrgan is injected; all urine voidings are collected separately again; and the time of onset of maximum flow and the length of time of the diuretic action of the drug are noted.

The third experimental period consisting of the second or postsalyrgan injection of theophylline, which is to show the combination effect of salyrgan followed by theophylline, is inaugurated. The control blood and urine samples are collected again. The same dose of theophylline previously used is injected intravenously and the time of onset of maximum flow and the length of the diuretic period are recorded. By comparison of these figures the accumulative effects are to be noted albeit roughly, but always definitely.

In some patients the salyrgan was given only a few hours before the theophylline and occasionally the drugs were used simultaneously.

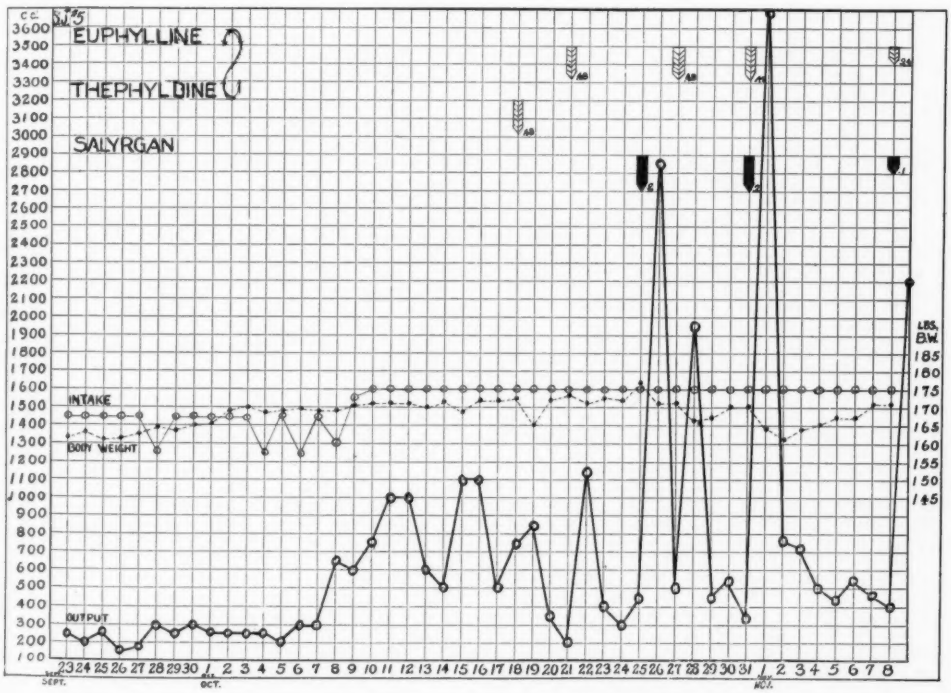

Fig. 5.—The daily record of a patient (S. J.) with hypertension coronary arteriosclerotic heart disease with congestive failure, cardiac cirrhosis of the liver and ascites. After a long rest period, two theophylline injections of 0.48 gm. each were given at three day intervals with but slight effect upon the urinary output. Salyrgan 0.2 gm. was then injected and a fair diuresis resulted. On the following day thephyldine produced twice as much of an output than it had previously. Four days later the drugs were given during the same morning a few hours apart and a good diuresis resulted, eight days later the drugs in half the dosage previously given had about two-thirds of the effect. The first was the only dose of euphyllin (the German product) the others were all thephyldine (the American preparation).

A fourth and fifth series of experiments were carried out with the hope of obviating the necessity of the intravenous injection of the drugs.

In the fourth series the patients were given a control injection of 0.1 gm. salyrgan or were put directly on one of purine derivatives, that is used for oral administration and the effects were noted and recorded. This drug is administered continuously three or four times a day according to the preparation used in doses of 0.1 to 0.5 gm. (1½ to 7½ gr.) until the output of urine was about at a constant baseline for three or four days. Then an injection of 0.1 gm. (1 c.c.) salyrgan was given and the rise in urinary output recorded. The

purine was continued through the mercurial day and for several days thereafter, and the results were charted.

In the fifth series the purine preparation was continued for a control period of several days and a mercury and chalk pill 0.0065 gm. (1 gr.) was given along with the purine by mouth three times daily for four to seven days and then the mercury administration was discontinued and the purine alone was continued for several more days.

RESULTS

Tables IV and V are so constructed as to represent a continuous story of the study of a patient. Each hospital day, the therapy and resulting fluid exchange is accounted for, reading from left to right and from the end of

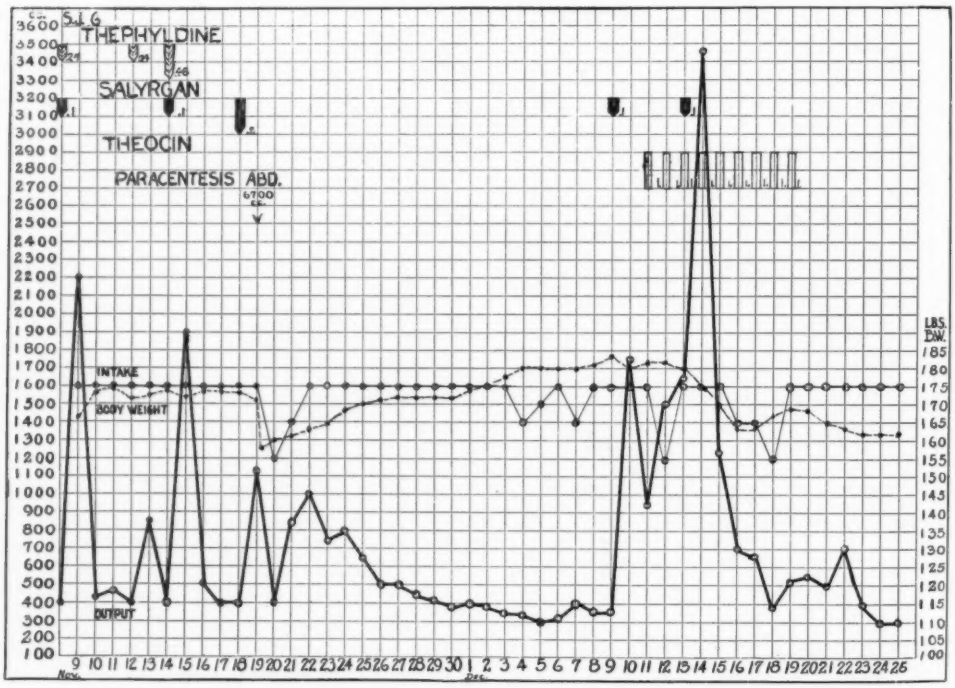

Fig. 6.—A continuation of the above record, showing the result of the last combination of half doses of salyrgan and thephyldine. A second combination with the full dose of thephyldine and the half dose of salyrgan was less effective than the previous half and half experiment, and following this the full 0.2 gm. dose of salyrgan resulted in but half of the urine output. Abdominal paracentesis was done and 6700 c.c. removed. Subsequently, 0.1 gm. salyrgan produced some diuresis but the same dose following three days of theophylline by mouth was doubly effective.

one line to the beginning of the next. The weight is frequently given as a rough index of the degree of or changes in edema and serves as a check on the results. It must be remembered that each succeeding diuretic experiment, especially when one succeeds another within a few days, begins under less favorable auspices for the edema in the tissue interstices has generally been reduced. The number of days of rest at the beginning and after each therapeutic attempt and the average urinary outputs per twenty-four hours for these days are recorded and taken as the baseline for calculations of the percentage of increase as the result of the drug administration.

TABLE I

TIME		INT	VOL	U	$\frac{U}{S}$ NaCl %	NaCl S %	CO_2	$\frac{U}{S}$ UR %	UR S %	$\frac{U}{S}$ CR %	S CR %	C	F	R	FU	U U	RU	UR %	EU %	U CL	F CL	R CL	E CL %	C CL
		INTERVAL IN MINUTES	URINE OUTPUT IN C.C.	RATE OF FLOW PER MINUTE	URINARY CL. MG. PER 100 C.C.	SERUM CL. MG. PER CENT	VOL. PER CENT IN BLOOD	URINARY UREA MG. PER 100 C.C.	SERUM UREA MG. PER 100 C.C.	CREATININE IN URINE MG. PER 100 C.C.	CREATININE IN SERUM MG. PER 100 C.C.	CONCENTRATION INDEX CR U %/CR S %	FILTRATION RATE GLOMERULAR C × U	REABSORPTION TUBULAR F − U	GLOM. FILTR. OF UREA $F \times \frac{U\ S\ \%}{100}$	URINARY EXC. OF UREA $U \times \frac{U\ U\ \%}{100}$	REABSORBED UREA F U − U U	PER CENT UREA IN R. $\frac{R\ U}{R} \times 100$	PER CENT OF F U EXC. $\frac{U\ U}{F\ U} \times 100$	UREA CONC. RATIO U U %/U S %	GLOM. FILTER OF CHLORIDES $F \times \frac{CLS\ \%}{100}$	URINARY EXC. OF CHLORIDES $U \times \frac{CLU\ \%}{100}$	REABSORBED CHLORIDES FCL.—UCL.	% CHLORIDES IN R $\frac{RCL}{R} \times 100$	% OF FCL EXC. $\frac{UCL}{FCL} \times 100$	CONCENTRATION RATIO OF CHLORIDES CLU %/CLS %
U*	7:05 A.M.																							
Bl	6:55																							
U	8:15	65	32	0.49	495	495	53	1330	25.0	200	1.62	123.5	60.7	60.2	15.2	6.55	8.6	14.3	4.30	53.2	305	1.03	304.0	565	0.336	1.00
Bl	8:15																							
U	9:25	70	56	0.80	363	545	50	923	28.6	445	3.34	133.0	106.5	105.7	30.5	7.38	23.1	21.9	2.42	32.3	581	2.91	578.1	547	0.500	0.666
Bl	9:25																							
U	9:15	70	52	0.74	363	528	52	857	33.4	407	2.50	162.7	121.0	120.3	40.8	6.34	36.5	30.0	1.55	25.7	639	2.69	636.3	528	0.421	0.688
Bl	10:55																							
U	10:45	90	56	0.62	429	561	53	857	20.0	445	2.50	178.0	110.7	110.1	22.2	5.32	16.9	15.2	2.40	43.2	656	2.67	653.3	592	0.407	0.764
Bl	1:00 P.M.																							
U	12:50	125	83	0.66	429	528	50	857	25.0	424	2.00	212.0	140.8	140.1	35.2	5.12	29.5	21.0	1.65	34.3	780	2.85	777.9	554	0.365	0.812
Bl	3:30																							
U	3:20	150	235	1.57	396	594	50	923	26.1	445	1.93	231.0	362.0	360.4	94.5	14.50	80.0	22.1	1.54	35.3	2150	6.25	2143.7	595	0.291	0.666
Bl	5:15																							
U	5:05	105	69	0.66	396	347	52	800	22.2	385	1.88	205.0	134.5	133.8	29.9	5.34	24.6	18.4	1.77	36.0	367	2.65	364.3	273	0.750	1.14
Bl	6:25																							
U	5:55	70	64	0.92	759	512	47	706	20.0	346	1.77	196.0	179.0	178.1	35.8	6.45	29.3	16.5	1.80	35.3	916	6.94	909.1	510	0.758	1.48
Bl	6:00 P.M.																							
U	-6:00 A.M.																							
Bl	8:00 A.M.	720	680	0.95	528	512	50	600	18.2	334	1.71	195.0	184.2	183.2	33.5	5.67	27.7	15.1	1.69	33.0	942	5.00	937.0	510	0.531	1.03

*U—Urine
Bl—Blood
S—Serum
Ur—Urea
Cl—Chloride
Cr—Creatinine (total)

TABLE II

	TIME	INT	VOL	U	NaCl U %	NaCl S %	CO_2	U U %	U S %	CR U %	CR S %	C	F	R	FU	U U	RU	UR %	EU %	CU	F CL	U CL	R CL	CL R %	E CL %	C CL
U	6:00-7:35																									
Bl	7:25	95	62	0.65	429	561	54	570	21.4	112.0	1.87	59.8	39.1	38.4	8.35	3.7	4.7	12.2	44.3	26.6	219	2.79	217.2	569	1.27	0.764
U	8:40																									
Bl	8:30	65	64	0.99	429	528	52	522	23.1	226.0	4.28	52.8	52.0	51.0	12.0	5.14	6.9	13.5	42.8	22.6	275	4.23	270.8	531	1.54	0.812
U	9:40																									
Bl	9:30	60	52	0.87	429	512	47	600	25.0	289.0	3.16	91.5	79.3	78.4	19.8	5.20	14.6	18.6	26.3	24.0	406	3.72	402.3	514	0.092	0.838
U	10:40																									
Bl	10:30	60	68	1.13	528	512	46	462	30.0	279.0	2.86	97.6	110.4	109.3	33.1	5.22	27.9	25.5	15.8	15.4	565	5.97	559.0	512	1.06	1.03
U	11:40																									
Bl	11:30	60	114	1.90	792	544	42	300	22.2	193.0	2.22	87.0	165.2	163.3	36.7	5.70	31.0	19.0	15.5	13.5	900	15.1	984.9	604	1.68	1.46
U	12:40																									
Bl	12:30	60	195	3.25	891	495	48	200	23.1	134.0	2.22	60.4	196.2	193.0	45.3	6.50	38.8	20.1	14.3	8.66	972	29.0	943.0	488	2.98	1.80
U	1:50																									
Bl	1:40	70	243	3.47	956	462	52	178	18.8	92.5	2.61	35.4	122.9	119.4	23.1	6.18	16.9	14.2	26.8	9.46	568	32.2	535.8	448	5.70	2.08
U	3:10																									
Bl	3:00	80	221	2.76	924	495	46	208	15.8	103.0	2.72	37.9	104.6	101.8	16.5	5.74	10.8	10.6	34.8	13.2	518	25.5	492.5	483	4.92	1.87
U	4:15																									
Bl	4:05	65	90	1.38	888	446	45	300	27.3	128.5	2.31	55.6	77.0	75.6	21.0	4.14	16.8	22.2	19.7	11.0	347	12.3	334.7	443	3.55	1.99
U	6:15																									
Bl	6:15	120	204	1.70	1386	363	46	184	27.3	105.0	2.31	45.5	77.4	75.7	21.1	3.12	18.0	23.8	14.8	6.74	281	23.6	257.4	340	8.4	3.82
U	6:00 P.M. to 8:00 A.M.																									
Bl	8:00 A.M.	720	1390	1.93	924	462	52	218	20.0	80.0	1.94	41.2	79.4	77.5	21.7	4.18	17.5	22.6	19.4	8.0	367	17.8	249.2	322	4.85	2.0

TABLE III

	TIME	INT	VOL	U	NaCl U %	NaCl S %	CO_2	U U %	U S %	CR U %	CR S %	C	F	R	FU	U U	RU	UR %	EU %	CU	F CL	U CL	R CL	CL R %	E CL %	C CL
U	6:00-7:00																									
Bl	6:50	60	49	0.82	363	577	53	333.0	15.0	115.0	2.61	44.1	36.0	35.2	5.40	2.71	2.7	7.68	50.0	22.2	205	2.96	202	573	1.44	0.63
U	8:20																									
Bl	8:10	80	53	0.66	693	544	48	387.0	14.6	193.0	6.0	32.2	21.4	20.7	3.12	2.56	0.56	2.78	82.0	26.5	1165	4.62	1123	542	0.397	1.27
U	9:30																									
Bl	9:20	70	757	10.8	428	528	48	132.0	14.3	74.0	3.16	23.4	253.0	242.2	36.2	14.3	21.9	9.5	39.6	9.23	1338	4.63	1333	550	0.347	0.81
U	10:30																									
Bl	10:20	60	980	16.34	396	512	49	113.0	12.0	73.5	3.0	24.5	400.0	383.7	48.0	18.5	29.5	7.7	38.6	9.40	2048	64.7	1983	517	0.316	0.773
U	11:30																									
Bl	11:20	60	1002	17.0	462	544	51	60.0	12.3	39.5	3.75	10.5	175.3	159.6	21.3	10.2	11.1	6.95	48.0	4.88	960	78.5	882	553	0.82	0.85
U	12:30																									
Bl	12:20	60	920	15.32	462	577	50	50.0	12.0	23.5	4.0	5.88	90.2	75.0	10.8	7.66	3.1	4.0	71.0	4.17	520	70.5	450	600	1.355	0.80
U	1:30																									
Bl	1:25	60	614	10.22	494	577	49	46.2	11.5	25.5	3.0	8.5	87.0	76.8	10.0	4.72	5.5	7.17	47.2	4.00	502	50.6	451	590	1.01	0.856
U	3:05																									
Bl	2:55	95	780	8.22	658	627		60.0	13.0	29.5	2.86	10.32	84.8	76.6	11.13	4.93	7.1	9.25	44.0	4.62	532	54.2	478	622	1.02	1.05
U	4:10																									
Bl	4:00	65	650	10.0	627	363		75.0	12.0	30.0	1.50	20.0	209.0	190.0	24.0	7.50	16.5	8.7	31.2	6.25	726	62.7	663	348	0.77	1.73
U	6:00																									
Bl	6:00	110	462	4.20	627	494		75.0	11.5	31.8	1.66	19.3	81.2	77.0	9.35	3.15	6.2	8.07	33.7	6.52	402	26.4	377	488	0.66	1.27
U	6:00 P.M. to 8:00 A.M.																									
Bl	8:00 A.M.	720	2400	3.34	658	494		26.1	11.2	44.5	1.57	28.36	94.7	91.3	10.6	1.28	9.3	10.8	12.1	2.33	467	22.3	445	490	0.478	1.33

If a spontaneous diuresis occurs or if the effects of a diuretic drug persist, the baseline will be high and the percentage of increase less striking. The percentage increase must therefore be considered with what has gone on previously and the actual urinary volumes should also be studied, and compared with the initial twenty-four-hour excretion amounts with full cognizance of the effects of previous diuresis. Therefore a direct comparison of percentage increases cannot be made. (See Figs. 5, 6, and 7.)

TABLE IV

THEOPHYLLINE IV AND MERCURY IV

	CONTROL REST PRELIMINARY			THEOPHYLLINE IV				REST POST-D. WITH T.			MERCURIAL IV			
	BODY WT.	DAYS	AVE. OUTPUT	DRUG	DOSE	RESULT	PER CENT INCREASE	BODY WT.	DAYS	AVE. OUTPUT	DRUG	DOSE	RESULT	PER CENT INCREASE
	lb.		c.c.		gm.	c.c.		lb.		c.c.		gm.	c.c.	
S. J.	173	2	250	EUP*	0.48	1060	325	172	3	330	S	0.2	2833	760
											S +	0.2		
	171	1	500	TPD	0.48	1814	263	170	3	425	TPD	0.48	3994	840
											S +	0.1		
								171	3	456	TPD	0.24	2230	389
											S +	0.1		
	174	3	433	TPD	0.24	915	112	174	1	400	TPD	0.48	1890	373
								174	3	430	S	0.2	1257	192
								183	20	498	S	0.1	1712	244
F. J.	132	3	320	TPD	0.48	1580	394	132	1	1100	S	0.2	4660	324
	132	2	525	TPD	0.48	4540	765	130	2	620	S	0.1	2431	292
	126	1	250	TPD	0.48	1150	360	125						
W.S.	170	3	525	TPD	0.48	1578	201	166	3	742	S	0.1	2522	241
	156	1	860	TPD	0.48	2682	213	150						
L. S.	146	7	320	TPD	0.48	953	197	146						
	146	2	1380	TPD	0.48	1696	23	145	1	1357	S	0.1	2737	102
	140	1	1050	TPD	0.48	4086	290	137	1	3173				
M.H.	149	S.D.												
	144	3	2020	TPD	0.48	2850	41	141	1	1960	S	0.2	3200	65
	141	2	1467	TPD	0.48	2212	51	140	2	1766	S	0.1	3316	89
	140	1	1955	TPD	0.48	1911	0	140	3	1280				
C. B.	155	4	395	TPD	0.48	875	122	153	1	1100	S	0.1	1800	64
	150	1	500	TPD	0.48	2500	400	144	9	650	S	0.1	2500	285
								137	13	714	S	0.1	3200	350
S. Q.	149	8	516	AMP	0.48	2104	314	140	1	1200	S	0.1	1203	0
	139	0	1203	AMP	0.48	2644	108	130	2	1120	S	0.1	1038	100

*Drugs:
EUP—Euphyllin, Theophylline ethlyene diamine.
TPD—Thephyldine, Theophylline ethylene diamine.
AMP—Aminophyllin, Theophylline ethylene diamine.
IV—Intravenously.
Post-D. with T.—After diuresis with a theophylline preparation.
T—Theophyllin.
S—Salyrgan.
S.D.—Spontaneous diuresis.

A glance at Table IV will reveal increases of from 100 per cent to 400 per cent with ranges mostly between 250 per cent and 350 per cent regardless of rises in the baseline output and decreased dosages. The greatest increase of 840 per cent was obtained in the fourth experiment in Case S. J. when the maximum doses of theophylline and salyrgan were given intravenously on the same day and the former preceding the latter by only a few hours.

In the fifth experiment three days later with the minimal doses for each

drug exactly half of those used in the fourth experiment a yield of 389 per cent increase was obtained. Three days later the half dose of theophylline alone intravenously gave a 112 per cent increased response and the next day the combination of the full dose 0.48 gm. of theophylline and 0.1 gm. salyrgan yielded a 373 per cent rise in output and three days after this 0.2 gm. salyrgan alone intravenously produced 192 per cent more than usual, and finally after twenty days' rest during which the body weight increased 9 pounds, an injection of 0.1 gm. of salyrgan enhanced the output 244 per cent.

The second case of F. J. shows the results just as strikingly as do also V. S., L. S., and C. B. Case 6, M. H., had a spontaneous diuresis at the very

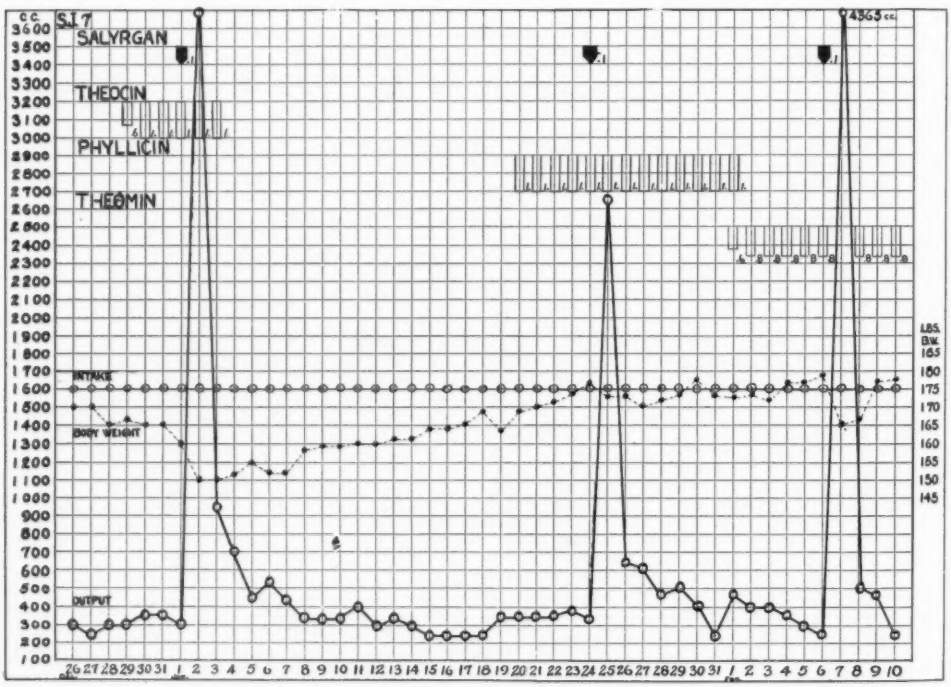

Fig. 7.—A continuation of the above record presenting a comparison of the diuretic effects of the combinations of theophylline in preparations by mouth before, during, and after injections intravenously of 0.1 gm. (1½ gm. or 1 c.c.) of salyrgan.

beginning which removed more than half of her total weight loss and therefore made the effects of the drugs much less striking. Case 7, S. Q., was treated with another preparation and at a lower dosage. Case 8, V. W., and 9, F. L .M., failed to respond to any of the diuretics and went to exitus with anuria following salyrgan.

Table V shows the results obtained in four patients, most of whom were repeatedly studied, during the use of a purine derivative alone by mouth over a period of three to six days then a mercurial usually salyrgan is added by injection on one day the results noted and the purine continued alone for several days. The results of the immediately preceding experiment are given first because most of these patients had been diuresed before the

TABLE V

PURINE P.O. AND MERCURY IV

	PRECEDING EXPERIMENT						PURINE P. O. ONLY				MERCURY IV + PURINE P. O.					PURINE ONLY DRUG			
	B W LB.	TREAT. DRUG	C.C. OUT.	AVERAGE OUTPUT			BODY WT.	DRUG	DAYS	AVE. OUTPUT	BODY WT.	DRUG	DOSE	RESULT	PER CENT INCREASE	BODY WT.	DOSE	DAYS	AVE. OUTPUT
		c.c.		Days	Drug	c.c.	lb.			c.c.	lb.		gm.	c.c.		lb.	gm.		c.c.
S. J.	173	S-0-2	1120	20	0	498	180	TC* 1.0	3	1333	182	S	0.1	3603	171%	175	TC 1.0	5	730
	157	Parac.	6700	1	S.0.1g.	1712													
				9	0	408	170	TC 1.0	4	330	165	S	0.1	4250	1190%	150	TC 1.0	1	940
				15	0	302	170	PC 1.0	5	360	173	S	0.1	2560	612%	170	PC 1.0	4	560
							173	TM 0.8	6	370	177	S	0.1	4300	1060%	166	TM 0.9	3	410
S. Q.							183	TBS 1.0	3	288	186	S	0.1	2850	890%	184	TBS 1.0	2	600
							193	TBC 1.5	5	507	192	S	0.1	2735	438%	191	1.5	1	1000
	145	Amp.	0.48 2644	1	0	850	140	TC 1.0	3	2066	122	S	0.1	2700	35%	117	TC 0.4	2	1100
C. B.	153	TPD	0.48 7500	5	0	1085	140	TC 1.0	4	862	150	S	0.1	2500	190%	143	TC 1.0	4	935
								TC 1.0	4	935	146	$HgCl_2$	0.0065	950	2%	145	TC 1.0	6	680
								TC 1.0	6	680	144	HgCN	0.0100	2500	268%	147	TC 1.0	2	650
								TC 1.0	2	650	142	S	0.1	3200	392%	140	TC 1.0	3	1065
E. D.				2	0	700	179	TC 1.0	4	750	179	S	0.2	2360	207%	177	TC 1.0	20	1055
							177	TC 1.0	20	1055	176	S	0.2	6800	339%	166	TC 1.0	1	1180

*TC—Theocin, Theophylline sodium salicylate
PC—Phyllicin, Theophylline calcium salicylate
TM—Theomin, Theophylline ethanolamine
TBC—Theobromine calcium salicylate
TBS—Theobromine sodium salicylate
S—Salyrgan

$HgCl_2$—Bichloride of mercury
HgCN—Cyanide of mercury
AMP—Aminophyllin
TPD—Thephyldine
Parac.—Paracentesis abdominis

"oral-purine-intravenous-mercury" régime was begun, and the facts must be taken into account in judging the results of the new procedure.

The average twenty-four-hour output of urine for a rest period, and the body weight at the end of this time constitute the basic levels. The average outputs are then recorded for several days during which a purine is given by mouth. Then the effect of adding an intravenous injection of usually 0.1 gm. salyrgan is noted and the purine alone is continued for several days. Reference to the table will show dramatic enhancement of the urinary excretion to more than 1000 per cent increase in several instances and usually a definite persistence of effects for several days in most cases. Although the experiments are comparatively few in number (12), there is such spectacular augmentation of urinary flow, agreeing so closely with our experience with intravenous administration of both drugs and our conceptions of the mechanisms of the two types of drug action that we accept them as complimentary evidence and feel no hesitancy in presenting them.

CONCLUSIONS

Clinical evidence seems to substantiate the idea that purine diuretics act primarily by increasing the glomerular filtration rate while the heavy metals accomplish their results principally by decreasing the tubular reabsorption.

Advantage was taken of these hypothetical considerations of the two different modes of action of the two types of diuretic drugs in the hope of getting better results by rational combination régimes.

Distinctly practical advances in the treatment of the vicious circle of edema in congestive heart failure are demonstrated.

The purines and heavy metal diuretics can be so combined as to (1) produce diuresis when either one of the drugs used alone in maximum dose has been ineffectual (2) as to accomplish the greatest possible diuresis in the shortest interval of time, (3) as to obtain a perfectly satisfactory diuresis by smaller and absolutely harmless doses which alone would prove inadequate.

Such complimentary effects, the result of combinations of the therapeutic action, even though hypothetically on different parts of the renal unit, are, we believe, of distinct practical value.

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PRIMARY CARCINOMA OF THE LIVER*

A Clinicopathologic Study

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THE liver is one of the organs in which secondary carcinoma occurs very frequently and primary carcinoma quite as infrequently. The malignant growths of the liver consist of epithelial tumors derived from the liver cells or the epithelial cells of the bile ducts, and mesenchymatous tumors originating either in the stroma or in the endothelium of the blood capillaries. The epithelial tumors are comparatively more frequent than the mesenchymatous ones. The purpose of this paper is to give a brief review of all the major contributions to this subject in the literature, and in this manner to establish as nearly as possible some idea as to its frequency. Further to call attention to some of the suggested conceptions concerning its histogenesis and finally to present analytical material, which is confined entirely to epithelial tumors, of 25 cases of primary carcinoma of the liver.

REVIEW OF THE LITERATURE

Prior to 1870, primary and metastatic carcinoma were not well differentiated. In 1876 Kelsch and Kiener[1] reported 2 cases, and at that time found only one other in the literature. Four more cases were added by Sabourin[2] in 1888 but the first thorough investigation was done by Hanot and Gilbert[3] later the same year. Von Hansemann[4] reported 258 cases of malignant tumor of the liver in 1890, 4 of which were primary.

Eggel[5] in his review of the literature up to 1901 reported 163 cases and one of his own. Among these only 117 were studied histologically. A summary of the reports up to 1916 by Winternitz[6] showed that the condition occurred in about 0.028 per cent of all necropsies. In a series of 3,700 postmortem examinations at the Johns Hopkins Hospital he found 3 cases. Goldzieher and von Bokay[7] in a series of 6,000 autopsies at the Pathological Institute of Budapest found 18 definite cases, an incidence of 0.3 per cent.

In Japan from the year 1904 to 1916 Yamane[8] collected a series of 57 cases found in 2,503 autopsies, an incidence of 2 per cent. Kika[9] in 1929 reported 110 cases found among 11,494 autopsies, and in a total of 20,185 autopsies in the Japanese literature since 1915, he found 223 cases, an incidence of 1.1 per cent.

Goldstein[10] in 1924 claims to have found, in the literature up to that time, about 250 cases of primary carcinoma of the liver as compared to 59 cases of sarcoma of the liver. Earlier in the same year Jaffé[11] reported a case of sarcoma and carcinoma occurring in the same liver. In the recent literature quite numerous reports of from one to several cases can be found. One obtains the impression in reviewing the subject that the condition is not as rare as is generally believed. Statistics of Orth[12] and von Hansemann[4] give about 0.5 per cent of all carcinomas as primary in the liver.

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It has been found in practically all ages and not uncommonly in children. Griffith[12] in 1918 compiled 57 cases in children ranging in age from one to sixteen years. In a baby girl of five weeks, Langmead[14] observed 8 pearly white tumors of the liver which proved to be carcinomas arising from bile ducts. Wollstein and Mixsell[15] reported a case in a child of four months. Twenty-three cases collected by Dansie[16] were under two and one-half years of age. In 1929 Kilfoy and Terry[17] collected 43 cases in children from one to sixteen years and reported one case of their own. Among the 43 cases 12 were proved by autopsy, the remainder were doubtful.

Karsner[18] concluded that primary carcinoma of the liver was most frequent in the fifth and sixth decades and this is supported by similar views from numerous investigators. As to sex incidence most writers agree that there is much more frequent occurrence in males. Geographically, those countries where liver infestations by flukes are common, report by far the highest incidence. From Japan, China, and South Africa evidences of this nature are quite numerous (Pirie,[19] Kika,[9] Yamane[8]).

CLASSIFICATION

There are two great classes into which neoplasms can be divided, namely, the benign and the malignant. Typical examples of these can be differentiated with no great difficulty, but between these two extremes there are intermediate grades in which certainly the reverse is true. A study of liver tumors brings out very clearly the truth of this statement. Ewing[20] in his book on "Neoplastic Diseases" draws attention to several interesting factors which are necessarily understood. "The regenerative capacity of the liver as exhibited in the destructive and inflammatory conditions furnishes necessary standards in estimating neoplastic processes in this organ. Extensive regeneration of necrotic areas is observed after focal lesions produced by hemolytic agents, and especially in acute yellow atrophy of the liver. Prolonged cases of this disease may show many islands of new liver tissue springing from the surviving liver cells in the periphery of the lobules and producing irregular masses of deformed lobules which may present a very bizarre and almost adenomatous appearance."

In addition to reparatory and regenerative processes, hamartoma, originally described by Albrecht[21] in 1904, has been considered by some investigators to have a definite relationship to true neoplasms. The term hamartoma is applied to growths that arise by reason of disturbances of development, as is indicated by the literal meaning of the word, "developmental error." Albrecht states they are only abnormal mixtures of normal structural constituents of the organ in which they occur, be it of the number, arrangement or grade of differentiation, or in all three respects. The idea has been well expressed by Warthin,[22] a part of whose definition bears repeating. "From the true tumors or blastomas there must be carefully separated tumor-like malformations or disturbances of development that show no autonomous excess of growth." He further intimates that such growths may form the basis for true neoplasms. Dyrenforth[23] states that it seems plausible, in view of the fact that many kinds of neoplasms have corresponding hamartomas, which at times are difficult to distinguish from them, to consider the preexistence of such a condition in some of the cases of primary carcinoma. Thus, a hamartoma of any organ might easily be conceived as antecedent to neoplastic change, for it is known that these representatives of disturbance of tissue do form bases for malignant tumors. He suggests that they are rarely found possibly because they frequently go unrecognized, for it is not necessary to the premise that they become malignant.

He compares the gross and microscopic appearance of two cases of hamartoma of the liver with a case of early primary carcinoma of the liver and brings out some points in common.

Concerning the histogenesis of these primary epithelial tumors Ewing states, "The growth of hepatoma from the hypertrophic liver cords has been observed by many writers. It has also been shown that there is a uniform gradation between nodular hyperplasia, multiple adenoma and multiple carcinoma. These gradations may even be observed in the same liver, so that as Muir has stated, there is no essential distinction between the comparatively benign and the atypical malignant forms of the tumor. During the transition the cells may retain the granular character of liver cells, while staining more intensely with basic dyes, or they may lose granules and pigment and assume the transparent, supposedly embryonal character described by Adler. Nuclear hypertrophy and hyperchromatism are very constant. Giant and multinucleated cells appear and mitoses and amitoses are frequent. Notable changes in the nuclei are often seen in the neighboring liver tissue, forming a feature of collateral hyperplasia. The first generation of tumor cells is usually large, later they may become smaller and atypical. The preservation and new growth of capillaries, whose endothelium resembles Kupffer cells, is a remarkable feature.

"Histologically there are two rather distinct varieties, one derived from the liver cells, the other from the bile ducts. As to the comparative incidence of these two varieties, there is nothing definitely settled among the writers who have committed themselves on this factor. Eggel[5] found 32 per cent of primary carcinomas of the liver to be cholangiomas and the remaining 68 per cent hepatomas. Pepere reduced the proportion of cholangiomas to 14 per cent. Yamane[8] found 79 per cent to be of liver cell origin. In the literature definite reports of bile duct carcinomas are much less numerous than cases of liver cell carcinomas."

The liver cell tumors are subdivided into three types.

1. A primary massive liver cell carcinoma. This type appears as a large single yellowish friable mass, often as big as a child's head, or occupying the whole right or left lobe. Small secondary tumors in the liver may be present. The consistency is usually soft and extensive liquefaction, necrosis and hemorrhage may produce a cystic appearance or rupture into the peritoneal cavity. The large veins are usually invaded and thrombosed. It is often associated with cirrhosis. Microscopically the structure presents wide variations, but always reveals at some points a definite resemblance to normal liver cells. In different portions four separate structures may be seen: (a) Trabecular adenocarcinoma composed of cords of very large granular acidophilic cells resembling large liver cells and separated by capillaries. Giant cells of very large dimensions are abundant in these areas. (b) Alveolar formation: These areas present compact groups of smaller granular epithelium sharply bounded by very numerous wide capillaries. (c) Peritheliomatous areas composed of capillaries surrounded by one or more rows of cuboidal granular cells. (d) Diffuse carcinoma: In many softened or necrosing areas all traces of the orderly arrangement of cells are lost, and the growth is composed of diffuse round polyhedral or spindle cells with strongly hyperchromatic nuclei.

These structural types cover most of those observed by other authors. It is evident that this tumor is a more rapidly growing, atypical and malignant form of the solitary adenoma.

2. Multiple liver cell carcinoma: In this group are included the highly malignant and rapidly growing tumors occurring in livers in which cirrhosis is absent or so slight as to be of secondary importance. There is, however, no sharp division between this group and the solitary massive carcinoma on the one hand and the multiple carcinoma following cirrhosis on the other. Here the liver is usually enlarged at times to very considerable dimensions and is the seat of multiple nodules and tumor masses which are grayish or bile stained or hemorrhagic and necrotic. Although the size of the sumor mass varies, it may be impossible to choose any one as a single primary focus. Many small nodules are doubtlessly secondary. Microscopically the structure varies greatly even in different portions of the same tumor and reproduces the more malignant features of the solitary carcinoma described above.

3. Carcinomatous cirrhosis: The tumor process appears to be a direct sequel of or essentially connected with cirrhosis. It shows an advanced stage of hyperplasia with atypical morphology, local aggressive properties and invasion of veins. The liver is usually contracted, but in some cases it is normal or somewhat increased in size. The surface presents multiple projecting yellowish or bile-stained nodules which on section may be found to represent a large part of the parenchyma. The nodules may be numerous, small and almost confluent, or larger, discrete and encapsulated. A portal cirrhosis is usually present, and many of the nodules are surrounded by connective tissue. Microscopic study shows extensive replacement of parenchyma by adenomatous and carcinomatous nodules. The tumor cells are of large size, forming cords, or the nuclei multiply actively and numerous smaller cells result. Peculiar forms of nuclear division, chiefly of the amitotic type are observed. Fatty degeneration may be prominent.

Biliary carcinomas have also been traced satisfactorily to the proliferating bile ducts. Precancerous changes are supplied by Milne and Yomagiwa in the form of angiocholitis proliferans. The multiple nodules must be referred to the universally distributed small ducts, while the solitary and cystic growths show some predilection for the hilus and subcapsular areas. This variety is less frequently associated with cirrhosis. The liver is usually enlarged. The dilated larger bile ducts may be visible in the gross. The tumor process affects most or all of the organ, and produces very numerous, usually small firm nodules, which may become confluent. The extensive hemorrhages, necrosis, the bulky soft tumor masses, and prominent invasion of large veins, which characterizes the so-called hepatoma, are missing. A tendency toward cicatricial fibrosis has often been noted.

The structural features usually form a sharp contrast with those of liver cell carcinoma. The cells are cylindrical, high or low or cuboidal, resembling those of bile ducts. The cytoplasm is clear, lacking the granular acidophilic character of liver cells, while the nuclei are small and vesicular. Mitoses may be numerous but giant cells are rare. The stroma is abundant and reveals the desmoplastic property of true carcinoma, while in the hepatoma the stroma is usually composed only of capillaries, although a scirrhous type is described, which is very difficult to dis-

tinguish from a cholangioma. Most of the nodules are enclosed in capsules of connective tissue, and the gradation into hepatic parenchyma seen in hepatoma is usually missing. The outlying portal canals commonly exhibit marked proliferation of the bile ducts, as in biliary cirrhosis. The arrangement of the cells in small alveoli is characteristic and at once distinguishes most of the tumors from the coarsely trabecular neoplasms, derived from the liver cells. In the slower growths the alveoli are regular and adenomatous. In the more malignant cases they become smaller, more numerous and irregular.

There is a division of opinion among the writers, as to whether liver cells arise from proliferating bile ducts or bile ducts from the liver cells. Certain carcinomas of the liver present areas which exhibit the characteristics of a hepatoma, with other areas in the same liver which show the structure of a cholangioma. It is suggested that in such cases a mixed type of tumor results from a neoplastic change affecting both these elements simultaneously (Orsos[24]). The most detailed study of the histogenesis in primary malignant tumors of the liver is that reported by Orsos, in which several types of carcinoma as well as sarcoma are exhaustively discussed.

MATERIAL STUDIED

In the pathologic records of the Cook County Hospital from June, 1928, until April 1, 1932, 19 cases of primary carcinoma of the liver were found. During this stated period 3,846 autopsies were performed. In the department of surgical pathology 2 cases have been proved by biopsy, during the years 1930 and 1931, making a total of 21 cases from this institution. From the Research and Educational Hospitals associated with the University of Illinois, the records of 4 cases were found during the years 1930 and 1931. In this period 198 postmortems were recorded. This makes a total of 25 cases in 4,044 autopsies, showing an incidence of 0.56 per cent. The postmortem examinations and histologic study of the 21 cases from the Cook County Hospital were made by Dr. R. H. Jaffé.

CLINICAL FEATURES

The clinical course of a patient with primary carcinoma of the liver varies greatly. Several well defined clinical groups are observed. (1) Cases in which no symptoms are detected and the patient dies suddenly from hemorrhage, after an illness of a few days. (2) Instances in which latent carcinoma is found in patients succumbing to cirrhosis of the liver and other diseases. (3) Cases with the usual history of cirrhosis terminating rapidly with hepatic tumor, jaundice, ascites and cachexia. (4) Lastly the cases with the usual history of a malignant tumor pointing from the first to the liver and developing in previously healthy subjects.

The ages of the patients ranged from twenty to seventy-three years, and about three-fourths of the total number of cases were in the fifth and sixth decades. Twenty-three of the patients were males, the remaining 2 females. Comparative racial incidence showed 21 white and 4 colored individuals. The clinical courses varied in length from six weeks to eighteen months.

Conclusions can be drawn from Table I concerning the clinical picture and postmortem findings in each individual case. In these it is interesting to note that in no instance was the diagnosis definitely made clinically. In three the possibility

TABLE I

CASES	RACE	SEX	AGE	CLINICAL FINDINGS: PAIN ABDOMEN	PAIN BACK	PAIN SHOULDER	RAPID LOSS OF WEIGHT	WEAKNESS	NAUSEA AND VOMITING	ANOREXIA	PALPABLE MASS	JAUNDICE	EDEMA OF ANKLES	FEVER	ANEMIA	DILATED ABDOMINAL VEINS	PALPABLE SPLEEN	DISTENTION	ALCOHOLISM	COURSE IN MONTHS	LIVER WEIGHT IN GRAMS	TYPE	POSTMORTEM FINDINGS: CIRRHOSIS	ASCITES	GALLSTONES	RUPTURE INTO PERITONEAL CAVITY	METASTASES: PORTAL VEIN	LYMPH NODES	LUNGS	ADRENALS	PAROTID GLAND	PERITONEUM	RIBS	VERTEBRAE	SKULL	ILIUM	CASES
1	W	M	69	+	+		+	+	+		+	+	+	+		+		+		11	2400	LC*	+	+		+	+									1
2	W	F	61	+	+		+		+		+	+		+	+		+		+	8	1510	LC		+				+	+		+					2
3	W	M	56	+	+		+		+	+	+	+		+	+			+	+	4	5390	LC	+	+		+		+								3
4	W	M	57	+			+			+	+	+	+		+					1½	4080	BD						+								4
5	W	M	57	+			+				+		+		+	+		+	+	1½	4810	BD		+				+		+						5
6	W	M	46	+			+		+		+	+		+						3	3570	LC	+	+			+									6
7	W	M	36	+			+	+			+	+	+					+	+	12	4050	LC		+				+								7
8	W	M	70	+	+						+	+	+			+				10	5660	LC	+	+	+				+	+		+			+		8
9	W	M	46					+			+	+						+		4	4380	LC	+	+			+		+								9
10	C	M	57								+	+			+			+		4	3460	LC		+			+										10
11	W	M	73	+							+	+						+		4	1480	BD			+												11
12	W	M	65	+			+	+			+	+			+				+	3	5020	LC			+												12
13	W	M	56	+	+		+	+			+	+						+	+	3	1995	LC	+	+			+										13
14	W	M	73		+		+	+			+							+		15	1390	LC	+	+				+	+	+							14
15	W	M	19	+		+	+	+	+		+			+	+	+		+		18		LC		+		No Autopsy											15
16	W	M	64	+			+	+	+		+	+	+					+		2	2565	LC	+	+			+										16
17	C	M	25				+		+	+	+	+		+			+	+		3	3280	BD		+			+		+								17
18	W	M	69	+			+		+	+	+	+		+	+					4	2000	BD		+													18
19	W	M	63	+		+	+	+	+	+	+		+	+	+	+		+	+	6	2460	LC		+				+	+								19
20	C	M	50	+			+	+					+		+					13	3620	LC						+	+		+						20
21	W	F	45								+	+			+	+				4	1030	BD		+				+	+					+			21
22	C	M	55								+		+					+		6	1425	LC		+													22
23	W	M	51	+			+	+	+	+	+	+		+	+	+		+	+	5	4020	LC	+	+					+								23
24	W	M	54	+			+	+					+	+						9		LC				No Autopsy											24
25	W	M	59	+			+	+			+	+		+	+	+			+	3	3860	LC		+			++			+	+		+	+	+			25
Totals				19	6	2	19	13	10	6	23	18	10	11	13	8	2	15	9				9	20	3	2	7	10	9	5	1	2	2	1	2	1	Totals
Percentage				76	24	8	76	52	40	24	92	72	40	44	52	32	8	60	36				36	80	12	8	28	40	36	20	4	8	8	4	8	4	Percentage

*LC, liver cell. BD, bile duct.

was mentioned. The clinical diagnosis was secondary or metastatic carcinoma in 10 cases, cirrhosis of the liver in 8, gall bladder disease in 2, organic heart disease in 2, and kidney tumor, carcinoma of the pancreas, and subphrenic abscess diagnosed once each. It is generally conceded that the symptoms and findings of malignant tumors of the liver do not differ materially, whether the growth be primary or secondary, carcinomatous or sarcomatous. In some instances there was no clinical evidence that the liver was affected. Pain, although it was by no means a constant symptom, was usually present at some period of the disease. It may be due to a stretching of the capsule or to a localized peritonitis. Occasionally it was more severe in the back than in the abdomen and infrequently referred to the shoulder. Among the 25 cases reported pain was present in 19, which to some degree and at some time was in the abdomen. In 6 it was felt principally in the lumbar region and two of the patients complained of pain in the shoulder. In these latter cases a solitary massive type of tumor was found in one lobe, which in both instances involved the diaphragm. Progessive loss of weight and strength was noted in all cases, but particularly was this true in the terminal stage. Jaundice occurred in 18 of the patients and in almost all cases was from mild to moderate in degree.

As nearly as possible an attempt was made to determine what symptom was first noticed by each patient. Seven complained of abdominal pain, 3 of lumbar pain and one of pain in the left shoulder. Of the remaining patients 5 first noticed a painless enlargement of the abdomen, 3 an anorexia, 3 a weakness, while dyspnea, edema of the lower extremities, and constipation were first apparent in one case each.

Progressive enlargement of the liver is perhaps the most important physical sign. A mass in the abdomen was palpated in 23 of the 25 patients. In 21 of these hepatomegaly was an outstanding finding. In 2 the liver enlargement was doubtful. The enlargement in one of these cases was thought to be a pancreatic tumor and in the other a renal tumor. It is also interesting to note that definite clinical signs of ascites was elicited in 18 patients or 72 per cent. Fever was found in 11 cases or 44 per cent. Eight of the patients showed dilated superficial abdominal veins or 32 per cent.

After the appearance of a malignant tumor of the liver, life is seldom prolonged over four months. Recovery is rare, but Keen[25] and Yeoman[26] reported successful excisions, some of which appeared to have recovered permanently. Six out of their 16 patients operated upon were alive and well from three to seven years postoperatively.

ANATOMICAL FEATURES

Grossly the livers varied in weight from 1030 to 5660 gm. As to consistency they were firm to hard, except when necrotic, and often showed thickened capsules. Only occasionally was there a decrease in size from the normal. Of the 23 cases which came to autopsy 4 livers weighed less than 1500 gm. The average was about 3 kilograms. The large solitary nodes, particularly, were often prone to hemorrhagic and necrotic changes. In two instances, there was a rupture of such nodes into the peritoneal cavity with fatal hemorrhage. On section, the nodes often presented alternating grayish pink to yellowish brown soft masses, penetrated here and there by white fibrous strands. Only a narrow edge of the liver parenchyma

remained of the lobe involved. In the multiple nodular type the liver substance was uniformly invaded by more or less sharply circumscribed reddish gray or bile stained nodules, varying from pinhead size to 6 cm. in diameter. In the carcinomatous cirrhotic type the desmoplastic reaction was more apparent, but there are transitions between these two latter types, making it difficult to differentiate them. Occasionally a liver will resemble externally an atrophic cirrhosis.

Six of the livers showed large solitary nodes with from few to numerous small intrahepatic nodules. Thirteen cases showed multiple nodular carcinoma and carcinomatous cirrhosis. The remaining 6 were cholangiomas. In these the nodules were usually ovoid and presented a rather colorful picture, the color ranging from whitish gray to brownish pink, sometimes bile stained, while the intervening liver tissue was brown, occasionally interspersed with yellow or yellowish green.

MICROSCOPIC FEATURES

The microscopic picture of the liver cell carcinomas reveals the tumor cells to be arranged in a very irregular fashion, often in interlacing strands, showing great variation in appearance. There may be some increase in the periportal connective tissue, which surrounds irregular islands of tumor cells, arranged in cords, but without grouping around a central vein. Some of the islands may present intercellular bile capillaries which are distended and filled by bile casts. Much bile pigment is occasionally found inside the liver cells. There are areas in which the cells are much increased in size with large hyperchromatic nuclei, undergoing direct division. Other areas are composed of irregular liver cells which form wavy bands and glandlike tubular structures, which not infrequently look strikingly like adenocarcinoma. The cytoplasm of these cells is homogeneous and oxyphilic. The nuclei are relatively small, round and rich in chromatin. In these areas no bile pigment is found. Many of the nodules show a tendency toward regressive changes and some of them are completely necrotic. Some nodes are composed of branched and anastomosing cellular cords, which are separated by thin strands of fibrillar connective tissue and capillaries. The cells which form the cords are polyhedral with an ample homogeneous slightly basophilic cytoplasm and round or oval nuclei. In the periphery of the nodes the tumor cells seem to pass into the cords of the adjacent liver tissue. By decreasing in size, assuming the basophilic coloration and enlargement of the nuclei the liver cells gradually become transformed into tumor cells. At times various sized and shaped cellular areas are separated by bands and regions of intercommunicating trabeculae of liver cells. The former areas are composed of finely granular light staining cytoplasm and large vesicular nuclei containing several oxyphilic nucleoli. In other cases they are seen in an alveolar arrangement, the alveoli being composed of round and polyhedral cells with distinct oxyphilic cytoplasm and round nuclei having a finely granular chromatin network. There are numerous mitotic figures, some being huge and markedly irregular. Giant and multinucleated cells are quite frequently seen. In the lumen of the portal capillaries and of the branches of the sublobular veins, tumor cells can be found. In the cells of the adjacent liver parenchyma much fat in the form of medium sized and large droplets is present. There are occasional erythrocytic extravasations.

The structure of the tumors arising from the bile ducts may also resemble that of an adenocarcinoma very frequently. The character of the tumor is tubular, and in the youngest areas is similar to proliferating bile ducts. There are narrow branched anastomosing tubuli lined by irregular cuboidal and, in some cases, columnar epithelium. The tubuli in a few instances become widened, and their lining reveals a tendency to form papillary infoldings. The papillae fuse together and thus finally the tubules result in a cell-filled alveolus. In the center of the alveoli there are at times extensive regressive changes. The cells are of irregular shape and size, and the anaplasia culminates into single huge elements with several bizarre nuclei. There is also a very abundant sclerotic stroma. In other cases the tumor is composed of dense fibrous tissue, in which single and groups of cells with large hyperchromatic and irregularly shaped nuclei and a relatively ample acidophilic cytoplasm are seen. Only small islands of liver tissue remain in most instances. The tumor nodules in many cases seem to be surrounded by fibrous connective tissue which separate them from the surrounding liver structure, whereas, in the so-called hepatomas there is rather a gradual merging of the tumor with the liver cells. Occasionally the picture may resemble very strikingly that of a hepatoma.

The frequency of metastatic growths is not great. They are principally hematogenous, although extensions to adjacent lymph glands and peritoneum is undoubtedly lymphogenous. Five of the cases reported showed no metastatic growths, and in four instances they were limited to branches of the portal vein. In the remainder of the cases the secondary growths were distributed in the lungs, adjacent lymph nodes, peritoneum, adrenal glands, parotid gland, meninges, ribs, spine, skull, and iliac bones. Table I shows the numerical distribution of the metastases in these various places. Secondary growths were found in the lungs in 9 of the cases and extension to the neighboring lymph glands was present in 10. Curiously, the adrenal glands showed metastases in 5 instances and in all cases the right gland was affected. In 3 of the cases bone metastases were discovered. Frequently the distant metastases of the liver cell carcinoma exhibited liver cell cords, Kupffer cells, capillaries, and bile secretion. This is important in that it offers proof of the origin from liver cells.

Of 163 cases tabulated by Eggel,[5] 46 were free from definite extensions, 50 showed growths limited to the portal or hepatic branches, 30 gave metastases to the lungs and thorax, 18 in regional lymph nodes only, and 9 secondary tumors were distributed in the colon, pancreas, ovary, kidney, omentum, thyroid, cranium, and brain. Moon,[27] in an article on bone metastases, reports the following sites: In the neck of the femur with spontaneous fracture, in the vertebrae, ribs, sternum and pelvic bones. Gadrat[28] reports a case with a secondary growth in the corpus cavernosum, producing a false priapism.

ASSOCIATED PATHOLOGY

There is little doubt that cirrhosis and the factors that lead to it cause degeneration followed by regeneration which may become excessive and neoplastic. It is one of the chief predisposing factors, occurring in about 85 per cent of the liver cell carcinomas and 50 per cent of the biliary duct tumors, according to Ewing.[20]

In our experience, however, the percentage is much less. Cirrhosis was found during postmortem examination in 9 cases of hepatomas and in none of the cases of cholangioma. Two of the cases were diagnosed by biopsy, and whether cirrhosis was present or not could not accurately be determined. This makes an incidence of 39 per cent of all cases, which is somewhat less than the reports of other writers.

Hemachromatosis associated with primary carcinoma of the liver has been reported in 14 instances or 9 per cent. Rosenthal[29] concludes that all the cases of hemachromatosis presented cirrhosis of the liver and that this relation of these two conditions does not predispose to carcinoma more frequently than cirrhosis of the liver alone.

SUMMARY

In conclusion, it suffices to say that in reviewing the literature it would seem that primary carcinoma of the liver is less rare than it has generally been believed. Certainly the highest incidence is reported in the Orient (2 per cent). The 25 cases reported here show an incidence of 0.56 per cent of all necropsies done at the Cook County and Research and Educational Hospitals, and 4.9 per cent of all malignant tumors. Relatively few cases of cirrhosis of the liver associated with primary hepatic carcinoma were found in this series of cases (39 per cent) which might indicate that perhaps it is not as important a predisposing factor as has been previously reported. Up until the present time, there has been little attempt to show that there are factors other than cirrhosis which are of importance in the origin of primary carcinoma of the liver. Evidence supporting the relationship which seems to exist between hamartoma of the liver and primary carcinoma of that organ is to a large extent convincing. It is urged that these malformations or developmental errors be sought for in the liver and studied with the thought in mind that many in the past may have gone unrecognized. In the mixed variety of tumors where the features of both the liver cell and bile duct carcinomas are found in the same liver, it is suggested that this is due to a neoplastic change affecting both these elements simultaneously. In most of the cases only one is affected resulting in a typical liver cell or bile duct variety. Attention is called to the difficulty in making the diagnosis of primary carcinoma of the liver clinically. Analytical data including a summary of the clinical histories, postmortem and histologic findings of 25 cases of primary carcinoma of the liver are reported.

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SWELLING OF THE EXTERNAL GENITALIA IN CASTRATED FEMALE BABOONS AFTER ORAL AND HYPODERMIC TREATMENT WITH FEMALE FOLLICULAR SEX HORMONE*

W. Schoeller, M. Dohrn, and W. Hohlweg

THE question of a definite follicular hormone dosage is still a problem. There has been considerable difference of opinion with regard to the size of the dose of follicular hormone in rodents. The view was held by many that the same dose which produces estrus, as measured by the Allen and Doisy test, is also the physiologic dose required to produce the complete phenomena of heat in mice or rats. Parkes[1] was the first to show that this is not the case. Normally, in sexually mature female rats, the vaginal changes occurring during the period of heat are also accompanied by hyperemia and increase in size of the uterus. An Allen and Doisy rat unit, while it admittedly produces an increase in the epithelialization and cornification of the vaginal mucous membrane, does not, however, suffice to cause a definite change in the uterus. Furthermore, the psychologic condition characteristic of heat is also only obtainable through the administration of larger doses than the Allen-Doisy unit. These results obtained by Parkes were confirmed in experiments carried out by Hohlweg and Dohrn.[2] They ascertained that the daily administration to adult, castrated, female rats of a dose of follicular hormone which was capable of producing permanent estrus (e.g., ½ to 1 rat unit daily) did not, however, suffice to prevent the castration changes in the anterior pituitary. To achieve this object it was necessary to give 3 to 6 rat units daily. From this work it will be seen that a daily dose which produces permanent estrus in mature female rats, and might thus be considered an overdose, is nevertheless insufficient to prevent another defect of castration and actually must therefore represent either too small a dose or an unsuitably spaced dosage. If then the drawing up of a scheme of physiologic hormone treatment for a species of animals such as the rat, about which so much is known, presents such difficulties, it will readily be seen that the determination of the physiologic dose of the hormone in human beings is a task of some magnitude.

For this reason we had already conducted earlier experiments on monkeys, which more readily allow of conclusions as to conditions in man. In these earlier researches[3] we were mainly concerned with demonstrating the peroral effect of the follicular hormone on female monkeys. We found that in the infantile female macacus rhesus 2.000 rat units administered over a period of a month in the form of pulverized tablets are sufficient to procure the full development of the previously infantile genital organs. The vagina,

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cervix, uterus, and mammae of the infantile animals show the same degree of development as is normally observed in mature females of the species during heat, i.e., at the time when the graafian follicle bursts and the maximum follicular hormone effect is observed. A comparison of the illustrations shown in the above mentioned treatise with those reproduced in the publication of Zuckermann and Parkes on female baboons clearly demonstrates this fact.

We conducted further tests on female baboons as in these animals an external change, i.e., swelling of the vulva, offers a method of estimating the effect of the follicular hormone. Furthermore baboons are more nearly related to human beings both in size and in the character of the sexual cycle than the macacus rhesus. The female baboon shows a fairly regular menstrual cycle which, just as in women, is accompanied by monthly bleeding. The normal cycle lasts on an average thirty days. The characteristic swelling in the genital region and perineum reaches its maximum at the time of ovulation. The swelling increases in size for seven to eight days, maintains its maximum degree for a few days and then rapidly declines (Zuckermann and Parkes,[4] Blotevogel[5]).

According to Zuckermann and Parkes the bleeding takes place twelve to fourteen days after the end of the period of maximum swelling. In castrated animals swelling does not occur. The appearance and disappearance of these typical signs of heat is therefore dependent upon the ovary and coincides almost exactly with the follicular phase. One could therefore assume that this change was produced by the follicular hormone and in actual fact Parkes and Zuckermann succeeded in reproducing this swelling artificially in castrated females by injection of the follicular hormone. Since these two English workers had confined their experiments to parenteral administration of the hormone, we resolved to investigate the question as to what peroral and intramuscular doses were necessary to reproduce swelling of the external genitalia in castrated female baboons.

Experiments on Peroral Administration.—In the first experiments, in order to make sure of obtaining a positive effect we gave the animal L (11.2 kilos) the high daily dose of 6.000 rat units progynon by mouth. We planned to continue the treatment for eight to ten days, in accordance with physiologic conditions. One gram of an extract from the urine of a pregnant animal containing 6.000 rat units was ground into powder with sugar and emulsified with 30 per cent alcohol. This mixture was poured into the animal's gullet once a day and followed by a drink of water so that for all practical purposes the entire quantity was taken. As early as the fourth day reddening of the nipples and genital region were already noticeable and the typical swelling quickly followed. At the end of nine days, during which the animal had received a total of 54.000 rat units, we suspended the treatment. The swelling reached its height on the second day following the last dose and was very marked.

In view of the definite reaction obtained in the first experiment, we conducted a second trial with only one-tenth of the previous dose, i.e., 600 rat units daily, administering this amount for eight days. The animal K (12

kilos) was employed and Figs. 1-A, 1-B and 2-A, 2-B show that in this case also a definite effect was obtained with a total dose of 4.800 rat units.

One month after the subsidence of the swelling the same animal was given 100 rat units daily for eight days and only a slight reaction was observed. From these three preliminary experiments we concluded that during an eight-day treatment about 6.000 rat units are necessary perorally in order to reproduce definite genital swellings in castrated female baboons, provided that a product is used which is active perorally. For the subsequent tests we employed progynon* tablets. In the fourth experiment the tablets were pulverized and administered in water. The animal J (weight 12 kilos) did not react to one and one-half tablets of 200 rat units given daily for eight days,

Fig. 1-A Fig. 1-B

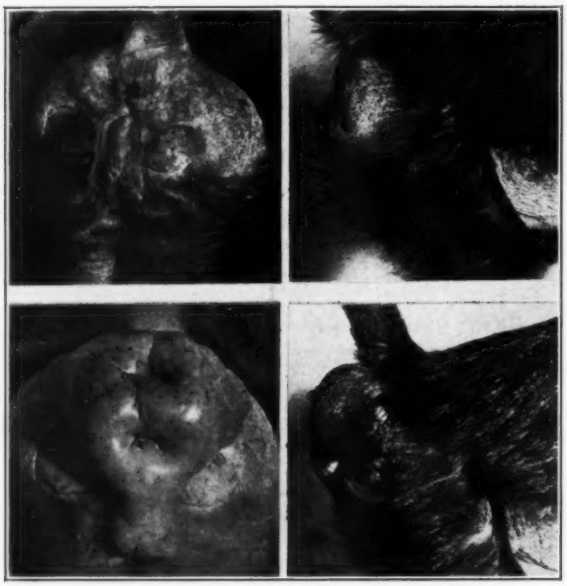

Fig. 2-A Fig. 2-B

while the animal K showed a good reaction with double this dose. In the fifth experiment we gave the tablets whole, and in this form, they proved equally effective.

The results of the sixth experiment were very interesting indeed. We had prepared tablets of 600 rat units each, and one of these tablets was administered daily for eight days to the three animals J, K, and E (weight 11 kilograms each). In this experiment the different reactive capacity of the three individual animals of nearly similar size was clearly demonstrated. J did not react at all, while K showed a moderate effect, and in E the results were very marked, as Figs. 3-A, 3-B, and 4-A, 4-B show.

In the seventh experiment the animal J, which had not reacted to 8 x 1

*Progynon is a preparation of the standardized female follicular sex hormone in tablet and ampule form. It is not reduced to the crystalline state as is the case in other preparations. The tablets contain certain lipoids of the placenta and ovaries in order to facilitate their absorption. The absorption of the tablets is on a ratio of one to five, one rat unit in five being absorbed when taken by mouth.

tablets of 600 rat units, showed marked swelling of the external genitalia after 2 tablets daily.

From these experiments it would appear that if our tablets are used about 6.000 to 8.000 rat units are necessary in order to produce the characteristic genital swellings in castrated female baboons provided the total dosage is spread over eight days.

Experiments on Intramuscular Administration.—Our trials with intramuscular administration of crystalline follicular hormone and its benzoate also gave interesting results. Butenandt[6] showed that progynon benzoate is capable of producing prolonged estrus in rats, and we were able to check his observations with a preparation made in our laboratory by Dr. Hildebrandt.

Fig. 3-A Fig. 3-B

Fig. 4-A Fig. 4-B

With a single dose of 0.25 mg. progynon benzoate estrus lasting on an average thirty days was produced in castrated female rats. Since the female ape has a longer follicular phase than the female rat, a longer excretion of the follicular hormone must be reckoned with on physiologic grounds. It was therefore to be expected that in female baboons a more marked difference in the effectiveness of the same dose would be observed, according to whether this was administered in a single dose or in divided doses and similarly according to whether a single dose of the free hormone or its ester was given. In the eighth experiment the animals E and M received an intramuscular injection of 5 mg. of crystalline follicular hormone* dissolved in 1 c.c. of oil.

*Crystals prepared from the urine of pregnant mares; melting point 245° C. Standardized on rats. The preparation dissolved in oil and injected in three portions spread over a day. Result: 400.000 rat units per gram. Judged by logical activity and the nature of the raw material, this preparation was fairly pure progynon, the strength of which is estimated by Butenandt at 300.000 to 400.000 rat units per gram.

Fig. 5-A Fig. 5-B

Fig. 6-A Fig. 6-B

Fig. 7-A Fig. 7-B

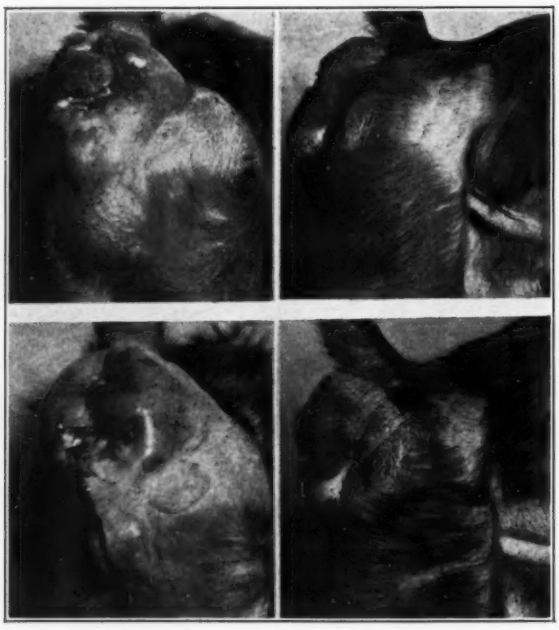

Fig. 8-A Fig. 8-B

Five mg. corresponded to 2.000 rat units. The animals J and K were injected with 6.925 mg. of progynon benzoate† dissolved in 1 c.c. of oil.

†Progynon benzoate, with a melting point of 215° to 216° C., prepared from crystals obtained in the manner above described.

TABLE I

SUMMARY OF EXPERIMENTS ON THE PERORAL ADMINISTRATION OF PROGYNON

EXPERIMENT	ANIMAL	PREPARATION	DURATION OF TREATMENT	DOSAGE IN RAT UNITS	RESULT
I	L	Progynon oil in alcoholic aqueous sugar solution	9 days	Daily 6.000 Total 54.000	Pronounced swelling
II	K	Progynon oil in alcoholic aqueous sugar solution	8 days	Daily 0.600 Total 5.400	Pronounced swelling
III	K	Progynon oil in alcoholic aqueous sugar solution	8 days	Daily 0.100 Total 0.800	Only very slight swelling
IV	K	Progynon tablets (200 R. U.) suspended in water	8 days	Daily 0.600 Total 4.800	Pronounced swelling
	J	Progynon tablets (200 R. U) suspended in water	8 days	Daily 0.300 Total 2.400	No swelling
V	K	Progynon tablets (200 R. U.) given whole	8 days	Daily 0.600 Total 4.800	Medium swelling
VI	J	Progynon tablets (600 R. U.) given whole	8 days	Daily 0.600 Total 4.800	No swelling
	K	Progynon tablets (600 R. U.) given whole	8 days	Daily 0.600 Total 4.800	Medium swelling
	E	Progynon tablets (600 R. U.) given whole	8 days	Daily 0.600 Total 4.800	Pronounced swelling
VII	J	Progynon tablets (600 R. U.) given whole	8 days	Daily 1.200 Total 9.600	Pronounced swelling

According to the standardization on rats, this dose of the benzoate corresponded in activity to the dose of free hormone given to the first two animals. The animals E and M, which were injected with the crystalline hormone, showed a very weak reaction on the fourth day after the injection and this rapidly disappeared. In the other two monkeys who received the benzoate, the swelling also commenced on the fourth day but increased considerably up to the seventh day, and remained constant for two or three days. Figs. 5-A and 5-B to 8-A and 8-B, which were all taken on the seventh day after injection, show the different effects of the free and the esterified hormone.

TABLE II

SUMMARY OF EXPERIMENTS ON THE INTRAMUSCULAR ADMINISTRATION OF PROGYNON

EXPERIMENT	ANIMAL	PREPARATION	DURATION OF TREATMENT	DOSAGE IN RAT UNITS	RESULT
VIII	E	Progynon cryst. 5 mg. in 1 c.c. oil	1 day	2.000	Slight swelling
	M	Progynon cryst. 5 mg. in 1 c.c. oil	1 day	2.000	Very slight swelling
	J	Progynon benzoate 6.9 mg. in 1 c.c. oil	1 day	2.000	Pronounced swelling
	K	Progynon benzoate 6.9 mg. in 1 c.c. oil	1 day	2.000	Pronounced swelling
IX	E	Progynon cryst. 2.5 mg. in 1 c.c. oil	8 days	Daily 0.250 Total 2.000	Pronounced swelling
	M	Progynon cryst. 2.5 mg. in 1 c.c. oil	8 days	Daily 0.250 Total 2.000	Pronounced swelling
	J	Progynon cryst. 2.5 mg. in 1 c.c. oil	8 days	Daily 0.500 Total 4.000	Pronounced swelling
	K	Progynon cryst. 2.5 mg. in 1 c.c. oil	8 days	Daily 0.500 Total 4.000	Pronounced swelling

The fact that the ineffectiveness of the 5 mg. of crystalline hormone was solely due to its administration in a single dose was clearly shown in the ninth experiment. In this experiment the animals E and M received the same dose as in experiment 8 but on this occasion the 5 mg. of crystalline follicular

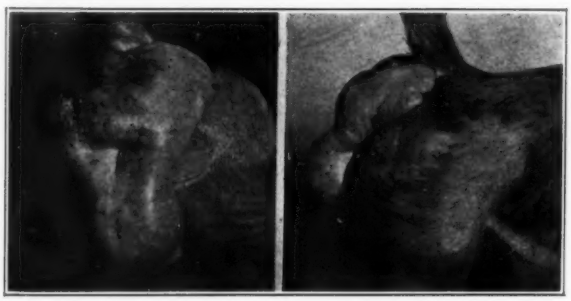

Fig. 9-A Fig. 9-B

hormone were spread over eight days. Both animals showed swelling of the external genitalia and Figs. 9-A and 9-B show the definite effect of the divided dosage (cf. Figs. 5-A and 5-B).

SUMMARY

The experiments show that with the peroral administration of 6.000 to 8.000 rat units of progynon spread over a period of eight days, the genital swellings characteristic of heat can be reproduced in the castrated female baboon. With subcutaneous injection of crystalline follicular hormone dissolved in oil 2.000 rat units spread over a period of eight days produced the same effect, while the same dose given in a single injection is inactive. If, however, progynon benzoate is used, a single injection of 2.000 rat units is sufficient to produce a definite reaction. Our investigations once again demonstrate that with a suitably chosen product and tablets of a particular composition, the peroral dose is only four to five times greater than the parenteral one.

In further researches which are at present in progress, we are endeavoring to bring about artificially the entire sexual cycle, including menstruation, in castrated female baboons, by preliminary treatment with follicular hormone, followed by the administration of corpus luteum hormone. The knowledge acquired in these experiments should then be sufficient to bring about menstruation in castrated women by combined treatment with these two hormones.

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THE ANTIDOTAL EFFECT OF SODIUM AMYTAL IN STRYCHNINE POISONING*

EDWARD E. SWANSON, INDIANAPOLIS, IND.

DAWSON and Taft[1] and Haggard and Greenburg[2] observed that several barbituric acid derivatives are antidotes in strychnine poisoning. Swanson[3] administered intravenously single equivalent effective doses of sodium amytal and pentobarbital sodium in rabbits poisoned with strychnine. In these experiments sodium amytal antidoted 6⅔ times the M.L.D. of strychnine and pentobarbital sodium 4⅙ times the M.L.D. of strychnine. Dawson and Taft[1] and Barlow[4] reported the injection of small effective doses of barbituric acid derivatives repeated at intervals as judged by the recurrence of strychnine convulsions. Clinically, in 1929 Zerfas and McCallum[5] found that sodium amytal when injected intravenously in single and repeated doses was an extremely effective and life-saving antagonist to strychnine poisoning. At the same time, rabbits poisoned with 2.5, 5, and 10 times the M.L.D. of strychnine were saved by single and repeated intravenous doses of sodium amytal (unpublished data).

This report is a continuation of the study of antidotal action of repeated small effective doses of sodium amytal in rabbits poisoned with strychnine.

Generally, it is agreed that 0.6 mg. per kg. of rabbit injected subcutaneously is the fatal dose of strychnine sulphate. In these experiments a 2 per cent solution of strychnine sulphate was used. Five and 10 per cent solutions of sodium amytal were used. The rabbit weights varied from 2.1 to 4.34 kg. Doses of 25 mg. and 12.5 mg. per kg. of sodium amytal were injected intravenously immediately following or during the first convulsion and repeated at intervals as required by the recurrence of severity of the convulsions. Injections of sublethal doses of sodium amytal made too rapidly or too frequently may result in respiratory failure and cardiac disturbance.

As shown in Table I, 12 mg. per kg. or 20 times the M.L.D. of strychnine require an average of 112.5 mg. per kg. of sodium amytal to save 4 out of 5 rabbits. With 15 mg. per kg. or 25 times the M.L.D. of strychnine, it requires an average of 121.5 mg. per kg. of sodium amytal to save 7 out of 10 rabbits. For 18 mg. per kg. or 30 times the M.L.D. of strychnine, an average of 137.5 mg. per kg. of sodium amytal is essential to save 6 out of 10 rabbits. Two of the surviving rabbits received artificial respiration at intervals during the treatment. Twenty-one mg. per kg. or 35 times the M.L.D. of strychnine require 143 mg. per kg. of sodium amytal plus artificial respiration to recover 5 out of 10 rabbits. Five rabbits injected with 24 mg. per kg. or 40 times the M.L.D. of strychnine fail to recover with 152.5 mg. per kg. of sodium amytal plus artificial respiration. Strychnine in such large doses probably exerts an additive depressive action with sodium amytal.

*From the Lilly Research Laboratories, Eli Lilly and Company.
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TABLE I

ANTIDOTAL EFFECT OF SODIUM AMYTAL IN RABBITS POISONED WITH SUBCUTANEOUS INJECTIONS OF STRYCHNINE SULPHATE

NUMBER OF RABBITS IN SERIES	STRYCHNINE SULPHATE MG. PER KG.	NUMBER OF LETHAL DOSES OF STRYCHNINE SULPHATE	SODIUM AMYTAL MG. PER KG.	NUMBER DIED	NUMBER SURVIVED
5	12	20	112.5	1	4
10	15	25	121.5	3	7
10	18	30	137.5	4	6
10	21	35	143.0	5	5
5	24	40	152.5	5	0

This shows that sodium amytal injected intravenously is antidotal to strychnine in 60 per cent of the rabbits poisoned with 30 times the M.L.D. of strychnine. Fifty per cent of the rabbits receiving 35 times the M.L.D. of strychnine recover with sodium amytal, together with the use of artificial respiration. With sodium amytal, it requires more to antidote equal amounts of strychnine than with pentobarbital sodium.[4] This difference in amount of sodium amytal is not twice that of pentobarbital sodium as would be expected, although the intravenous M.L.D. of pentobarbital sodium in rabbits is 40 mg. per kilogram and that of sodium amytal 80 mg. per kilogram (unpublished data). The real difference, apparently, is the duration of action. With equal effective doses of sodium amytal, the duration of action is obviously longer than that of pentobarbital sodium. Thus during the critical period of strychnine poisoning, sodium amytal is less frequently required.

CONCLUSIONS

Sodium amytal by vein in small repeated doses antidotes 20, 25, 30, and up to 35 times the subcutaneous M.L.D. of strychnine sulphate in rabbits.

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PRIMARY SARCOMA OF HEART*

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THE heart is peculiarly immune to neoplasm, and particularly to primary tumor. It is therefore deemed noteworthy to report a case which came to autopsy at the City Morgue in Brooklyn, New York, Kings County Hospital: a case of sudden death upon the street, and reported to the Medical Examiner's Office in that Borough of New York City.

The completion of a routine autopsy performed on this case proved it to be one of unique interest. Investigation of the literature anent primary cardiac neoplasm has instigated this report. Through the courtesy of Dr. Manuel E. Marten, Deputy Chief Medical Examiner of Brooklyn, we are enabled to report and place this case on record.

This is a case (W. P.) of a well-developed, muscular negro, aged fifteen, 5 feet tall and weighing about 135 pounds.

Family History.—The father was a sailor and apparently in good health; the mother was also living and well. She admitted only one pregnancy, that of the deceased. Delivery was normal, and at the time of birth no abnormalities were noted.

Physical History.—He was subject to frequent colds and sore throats, syphilis and gonorrhea were denied, had no exanthemas, had been subject to chronic bronchitis, had had no operations or hospital treatment, no addiction to alcohol or drugs, appetite was good, slept well, mode of life was within normal limits apparently, no history of neurologic manifestations or systemic disorders of any nature.

Present Illness.—He had been employed as an errand boy, attended night school and participated in gymnastics, and occasionally attended social functions such as dances. On the night of his death, while on the way to a party, he complained to a companion of feeling ill and started for his home, when he collapsed in the street from sudden illness. An ambulance was summoned, and upon its arrival he was pronounced dead. The body was removed to the City Morgue at Kings County Hospital for determination of cause of death, and necropsy showed cardiac neoplasm. Upon further investigation it was ascertained that about a year and one-half before his death he began to complain of dyspnea and cardiac palpitation on exertion, and was treated by a physician as a cardiac. No history of cardiac symptoms is available that may have existed prior to his having come under the doctor's care. His health having been uniformly good prior to his cardiac history naturally curtails the clinical history in this case.

*From the Pathological Laboratory of Kings County Hospital, William W. Hala, M.D., Director.

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At necropsy the following findings were established:

Head: Scalp and skull negative.

Brain: Subpial congestion and edema.

Thorax: Heart weighed 1325 gm. There was a tumor-like mass in the right auricle, filling the entire chamber and consisting of soft, fat-like material interlaced with coarse fibrous strands. The mass was 6¼ inches in its greater diameter and 4 inches in the lesser, and correspondingly distended the auricle (Figs. 1 and 2). The wall of the auricle averaged ¼ inch in thickness, and

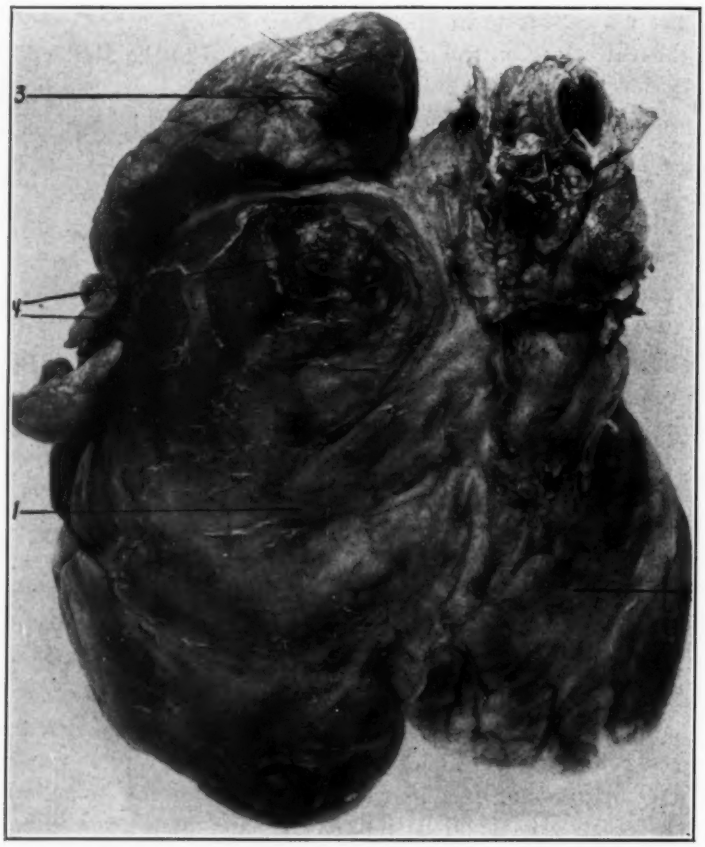

Fig. 1.—This picture illustrates the enormous size of the right auricle distended by the tumor within it. *1*, Right auricle. *2*, The rest of the heart, including the left auricle and both ventricles. *3*, Right lung. *4*, Points of rupture which occasioned the sudden death and the patient.

showed at its upper border 2 ruptures, one 1¼ inches in diameter and the other ¾ inch. Both openings were covered with blood clot. The left auricle appeared normal. The combined width of the ventricles was 3¾ inches by 3½ inches, and the chambers were normal. The musculature was of good quality, and the valves and the orifices were grossly normal. The aorta was normal except for numerous atheromatous plaques on the intima.

Right Lung: Weighed 480 gm.; was displaced by the enlarged auricle and compressed to about three-fifths the normal size; dissection of the lung showed

atelectasis of the middle lobe and slight exudation of frothy fluid from the upper and lower lobes.

Left Lung: Weighed 510 gm.; on section, frothy fluid was expressed from the cut surfaces.

Right Pleural Cavity: Contained about 1½ quarts of fluid and blood clot. The right auricular wall was adherent to the pericardium at the points of rupture, and the fluid and clotted blood had escaped through the pericardium into the right pleural cavity.

Fig. 2.—Anterior view of the heart and the enormous auricular tumor. The growth, in size, greatly exceeds the rest of the heart. *1*, Right auricle. *2*, The rest of the heart. *3*, Right lung. *4*, Trachea. *5*, Points of rupture.

Abdomen: Liver: Weighed 1300 gm. On section showed chronic passive congestion only.

Spleen: Weighed 40 gm. The capsule was tense and slate blue in color. On section, the pulp was firm and the follicles prominent.

Kidneys: Combined weight 250 gm. The surfaces were reddish brown in color and engorged. No gross pathology was found on section. The ureters and urinary bladder showed no changes.

The pancreas, adrenals, gastrointestinal tract, and the remaining viscera were grossly normal.

Examination of the bones showed no gross changes.

The histologic examination of sections taken from the auricular tumor revealed a histoid neoplasm composed exclusively of small round cells of lymphocytic morphology. There were areas of necrosis which accounted for the peculiar yellowish (lipoidal) appearance of the tumor, and which on gross examination caused a provisional diagnosis of liposarcoma.

Primary tumors of the heart are rare. Usually cases are demonstrable at autopsy only, since the diversity of clinical manifestations, involving, as they do, a variety of lesions, give no specific indication of cardiac tumor. It may therefore be stated that neoplasms of the heart are of greater interest to the pathologist because of their objectivity, than to the clinician. On the other

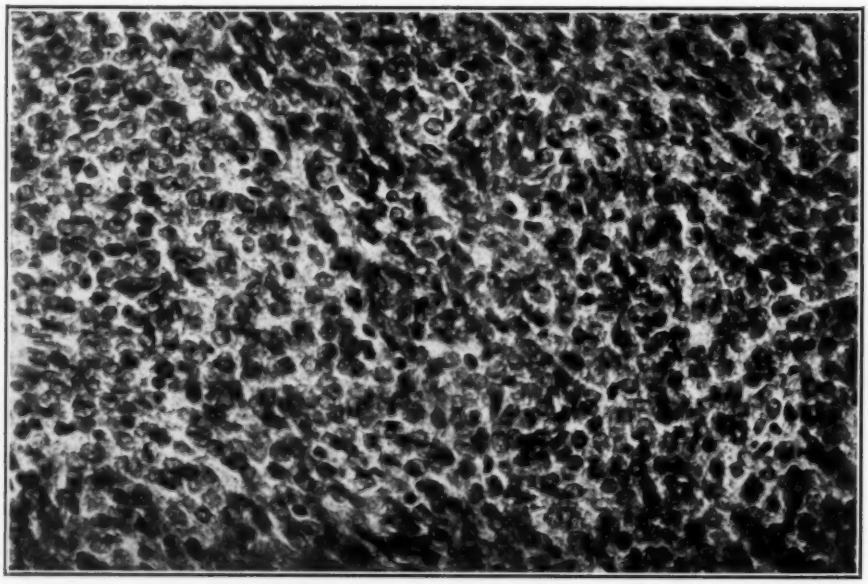

Fig. 3—The histologic architecture of the tumor is well shown in this photograph. It is entirely histoid and consists entirely of small round sarcoma cells within a delicate meshwork of reticulum.

hand, review of the literature would seem to indicate the possibility of identification of primary cardiac tumor during life, although nothing specific appears to be adduced, to substantitate this.

Metastatic involvement is of course of far more frequent incidence, and its existence in the heart is inferred because of the determination of a primary focus elsewhere. The origin of such metastases has been reported from melanotic sarcoma of eye, and cancers of the breast, mediastinum, stomach, adrenals, uterus, and penis. The majority of these secondary tumors of the heart are carcinomatous in type rather than sarcomatous.

In the premicroscopic days Morgagni,[1] in 1762, described multiple polypoid tumors of the heart, but as to whether these were intramural thromboses or true neoplasms is, of course, speculative. The first authentic demonstration of primary cardiac tumor was that of chondroma by Albers,[2] in 1835, and di-

agnosed histologically as fibroma. The first malignant connective tissue tumor was reported by Bodenheimer[3] in 1865.

More than 100 cases have been reported of primary tumor of the heart, but many of these have been rejected by histopathologic findings and classed as metastases or pseudotumors. Tedeschi[4] reviewed 86 cases, including 3 of his own, many of which were secondary tumors.

Berthenson[5] found 30 cases of primary tumor, 7 of which were sarcoma. Ehrenberg[6] collected 19 cases of sarcoma of the heart. Link[7] reported 91 cases, of which only 23 were of value from a clinical standpoint, and 13 specifically demonstrable as sarcoma. Up to 1877 Bodenheimer collected 7 cases of sarcoma of the heart, which had been found by Ely[8] in fifty years of the literature. Leyden and Fraenkel reported 2 cases, which were included in Berthenson's summary. Of these 9 cases the round cell predominated in 8, the other being of giant cell type. Bernheim in 1890, reported a sarcomatous polyp of the right heart. Hektoen[9] reported a case of primary sarcoma of epicardium, and Bryant reported a similar lesion in the heart of a dog.

In 1896 Leroux and Mesely reported a primary sarcoma of the heart. Raw and Lambert reported[10] Raws' case, a spindle-celled sarcoma of the right auricle; Lambert[11] a primary angiosarcoma of heart and pericardium, described by Redtenbacher.[12] Azzuni, 1907, added another case of heart tumor. Baldwin[13] reported one case of primary large spindle-celled sarcoma of heart, making a total of 17, to which may be added our own case: 18 in all. Except for a case reported by Armstrong and Monckeberg,[14] which was a lymph-angio-endothelioma, all cases were sarcomatous in origin.

A. Matras,[15] April, 1927, reports from the literature 26 cases of sarcoma of the heart, 13 of which originated in the right auricle, mostly in the auricular septum: 1 case in both auricles, 2 in the left auricle, 3 in the right ventricle, 2 in the left ventricle, 1 in the endocardium of the aortic valve, 2 in the ventricular septum, 1 in the endocardium of the pulmonary valve, and 1 case the origin of which could not be determined because of the diffuse involvement of the entire heart. Perlstein[16] reports a case of cardiac sarcoma, originating in the subpericardial areolar tissue of a male forty-three years old. Adami states that cardiac neoplasm is rare due to activity, efficiency nourishment and innervation.

Heart tumors most commonly encountered are fibromas and myxomas. Next in incidence are sarcoma. Joel[17] reports a teratoma. There also have been reported rhabdomyomas, lipomas, angiomas, lymphangiomas. Rhabdomyomas are usually congenital and in about 50 per cent of cases are associated with a tuberous sclerosis of the brain.

Of 2,942 necropsies at Johns Hopkins Hospital, only 10 heart tumors were found, all of which were secondary except 1 which was a rhabdomyoma, the remaining 9 being: sarcoma 1, lymphosarcoma 1, epithelioma 2, endothelioma 1, and 4 carcinomas (Bryant[18]). From 1903 to 1907, 6,655 necropsies performed at the University of Berlin Pathological Institute revealed 15 secondary carcinomas and 4 secondary sarcomas of the heart (Karrenstein[19]). In the past

decade, in over 9,000 cases brought to autopsy at Kings County Hospital Morgue, the case herein described is the only one of primary sarcoma of the heart.

Thorel reports that in over 3,000 autopsies at Nuremberg, no cardiac neoplasm was seen.[20] The records at Bellevue Hospital[21] in over 7,000 cases show no cardiac primary neoplasm.

SUMMARY

This instance of primary sarcoma of the heart is reported in a case which unfortunately furnishes but little clinical history. The tumor is a small round-celled sarcoma, originating in the right auricle, which because of the apparent normal health of the deceased in almost fifteen years of his life, must have been insidious in onset, but after that a rapidly growing fatal neoplasm.

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THE EFFECTS OF ADRENALECTOMY ON THE CARDIAC OUTPUT AND BLOOD PRESSURE*

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THE alterations in the cardiac output and blood pressure following a number of procedures that result in acute circulatory failure have been determined. Blalock[1] found that the removal of blood from dogs caused a marked decrease in the output of the heart before a significant decline in the mean arterial pressure occurred. Similar findings were obtained by Johnson and Blalock[2] in experiments in which shock was produced by trauma to an extremity, by trauma to the intestines and by burns. Following the subcutaneous injection of histamine, the alterations appeared in the reverse order, there being first a drop in the blood pressure followed later by a decline in the cardiac output. The effects of trauma to the central nervous system were studied by Blalock and Bradburn.[3] The findings in these experiments were inconstant but usually the cardiac output and blood pressure declined simultaneously. The effects of spinal anesthesia were determined by Burch and Harrison.[4] They state, "From the data it appears that in spinal anesthesia the initial change is, as would be expected, in arterial pressure, and that the venous return and output of the heart are affected secondarily. This sequence of events is just the opposite of that in hemorrhage."

The present experiments were performed in an endeavor to obtain information concerning the nature of the circulatory failure that develops following the removal of the adrenal glands.

METHODS

Dogs were used in all experiments. The operations were performed in two stages under ether anesthesia through lumbar incisions. The right adrenal gland was removed first. Approximately two weeks following the first operation, the dog was given one-half grain of morphine and an hour later the cardiac output and blood pressure were determined. The left adrenal gland was then removed. The animals were kept warm and observed closely. Additional determinations of the cardiac output and blood pressure were performed after varying intervals of time. When the animals were not quiet, morphine was given prior to the studies.

The arterial pressure was measured by a mercury manometer, the cannula being inserted into the femoral artery. Valves were used for determining the maximum and minimum pressures. The cardiac output was determined according to the Fick principle. Arterial blood was withdrawn from the femoral artery and mixed venous blood was obtained by puncture of the right ventricle. The Van Slyke-Neill apparatus was employed in determining the oxygen con-

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tent. The oxygen consumption was determined by the use of a Benedict spirometer. The blood that was removed for the studies was replaced by an equal amount.

RESULTS

Nine experiments were performed. In seven of the nine experiments, a rather marked decline in the blood pressure preceded any significant alteration in the cardiac output. In the remaining two experiments both the blood pressure and cardiac output had declined at the time of the second determinations. In three experiments in which early alterations in the blood pressure were detected, there was first a definite decrease in the maximum and a slight increase in the minimum pressures. This was associated with an increase in the pulse rate. The behavior of the animals differed from those previously studied in which shock was produced in various ways, in that the present animals appeared to be in fairly good condition and lived for a considerable period of time after the blood pressure had declined to a rather low level.

The results of a representative experiment are given in Table I.

TABLE I

THE EFFECTS OF ADRENALECTOMY ON THE CARDIAC OUTPUT AND BLOOD PRESSURE

TIME	WEIGHT KG.	ARTERIAL O_2 VOL. PER CENT	VENOUS O_2 VOL. PER CENT	A. V. DIF. VOL. PER CENT	MAX. B. P. MM. HG.	MIN. B. P. MM. HG.	O_2 CONSUMPTION PER MIN. C.C.	CARDIAC OUTPUT PER MIN. C.C.
Control studies	15	12.61	7.22	5.39	145	81	121.0	2245
27½ hours post-op.		11.68	6.99	4.69	99	61	114.6	2443
28½ hours post-op.		12.76	7.86	4.90	99	61	120.5	2459
33½ hours post-op.		14.18	6.74	7.44	82	64	109.8	1476
40 hours post-op.		13.52	5.98	7.54	59	45	101.1	1341
45½ hours post-op.					45	39		

Protocol: Oct. 7, 1931, right adrenal gland removed. Nov. 11, 1931, 11 A.M., given ½ grain of morphine. 12 noon, cardiac output and blood pressure determined. 5 P.M., left adrenal gland removed, ether anesthesia. Nov. 12, 1931, 7:45 P.M., given one-half grain of morphine. 8:30 P.M., and 9:30 P.M., determinations. Nov. 13, 1931, 2:15 A.M., determinations. 9 A.M., given ¼ grain of morphine. 9:30 A.M., determinations. 2:30 P.M., blood pressure determined. 2:45 P.M., dog died.

DISCUSSION

The removal of both of the adrenal glands of dogs is followed by alterations in the cardiac output and blood pressure that are similar to those produced by the subcutaneous injection of histamine and by spinal anesthesia and probably to those occurring in primary shock. Following adrenalectomy both the output of the heart and the arterial blood pressure may remain at the normal level for a considerable period of time. Rogoff and Dominquez[5] found that the blood pressure following the removal of both adrenals remains elevated until several days preceding death. Their animals had a longer survival period than ours. This is due in part to the fact that the animals in the present experiments were given morphine from time to time in order to have them quiet during the studies. Morphine usually caused vomiting and diminished the amounts of food and water that were taken. Burwell and Smith[6] found that the low blood pressure in a patient with Addison's disease was associated with an essentially normal cardiac output.

Concerning the early deaths following adrenalectomy, Rogoff and Stewart[7] state, "As stated, we think that when dogs die in two or three days or less something else than adrenal insufficiency has contributed to shorten life, especially the direct consequences of the surgical procedure." If this is true, the operative shock is associated with alterations in the cardiac output and blood pressure unlike those accompanying hemorrhage, trauma to the intestines, trauma to an extremity, and burns.

SUMMARY

The effects on the cardiac output and blood pressure of dogs of the removal of the adrenal glands have been studied. The initial alteration consisted of a definite decline in the arterial pressure followed later by a decrease in the output of the heart.

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LABORATORY METHODS

A NEW BLOOD PLASMA CHLORIDE METHOD*

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MERCURIC halides are very slightly dissociated and, due to this fact, several attempts have been made to develop a mercurimetric method for the determination of chloride. Little success was met with until Votoček[1] introduced sodium nitroprusside as an indicator.

When a standard mercuric nitrate solution is added, the chloride ions are removed as soluble, non-ionized mercuric chloride. After the removal of the chloride ions, white mercuric nitroprusside, which is insoluble in acid solution, is formed, thus producing a turbidity which indicates the end point.

Kolthoff and Bak[2] showed that the sensitivity of the indicator is reduced in the presence of the slightly dissociated mercuric chloride and gave the titration corrections to be used with various concentrations of mercuric chloride in water solutions.

Recently Filedt Kok[3] applied the method to the determination of chloride in blood and serum, and recommended the use of sulphosalicylic acid as a precipitant for the proteins. The Folin-Wu filtrate was rejected because the reagents used to prepare the filtrate appeared to contain chlorides. The contamination of the filtrate is not obtained when Merck's C. P. sodium tungstate is used. Peters and Van Slyke[4] give a method for the testing and purification of the reagent.

It appeared that the above procedure, if suitably modified, would have certain advantages in the clinical laboratory over the standard methods now in use.

METHOD

A Folin-Wu filtrate of the blood or plasma is prepared (1 c.c. of blood or plasma, 7 c.c. of water, 1 c.c. of 2/3 *N* H_2SO_4 and 1 c.c. of 10 per cent sodium tungstate are thoroughly shaken together and filtered).

Five cubic centimeters of the filtrate are pipetted into a large test tube and 0.2 c.c. (3 drops) of fresh 5 per cent sodium nitroprusside solution are

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added. Mercuric nitrate* (1 c.c. is equivalent to 1 mg. NaCl) is added from a micro-burette until a permanent turbidity is produced on the addition of one drop.

(Titration value in c.c. – X) × 200 = mg. of NaCl per 100 c.c. plasma

The titration corrections (X) which Kolthoff and Bak determined for varying concentrations of $HgCl_2$ in water are slightly low for the Folin-Wu filtrate. Accordingly, these factors have been redetermined for the Folin-Wu filtrate and are recorded in Table I.

TABLE I

5 C.C. OF FOLIN-WU FILTRATE		10 C.C. OF FOLIN-WU FILTRATE*	
TITRATION VALUE IN C.C.	TITRATION CORRECTION X	TITRATION VALUE IN C.C.	TITRATION CORRECTION X
1.5	0.07	3	0.14
2.0	0.08	4	0.16
2.5	0.09	5	0.18
3.0	0.10	6	0.20
3.5	0.11	7	0.22

*Should 10 c.c. of the filtrate be titrated the formula becomes (titration value in c.c. - X) × 100 = mg. NaCl per 100 c.c. of blood or plasma.

DISCUSSION

A number of samples of blood and serum have been analyzed by this method and check analyses made by the Whitehorn method, which was modified to the extent of centrifuging out the AgCl before titrating to the end point. The average variation of the results of the two methods on the different samples was 7.2 mg. NaCl per 100 c.c. of blood or plasma, with no regularity as to which method gave the higher result.

Accurate results may be obtained on 5 c.c. of a filtrate prepared from 9 c.c. of 2½ per cent sulphosalicylic acid and 1 c.c. of blood or plasma if the mixture is thoroughly shaken before filtering. However, the end point is slightly less distinct than with the Folin-Wu filtrate, as there is a tendency for the sulphosalicylic acid solution to form small bubbles when shaken. If centrifugation is used to separate the precipitated proteins from the filtrate, excellent results are obtained when the Folin-Wu precipitation is used, but a slightly turbid solution is obtained with the sulphosalicylic acid. Based on this observation the chlorides in 0.2 c.c. of finger tip blood can be determined in the following manner:

One and eight-tenths cubic centimeters of tungstic acid solution (7 volumes of water, 1 of sodium tungstate, and 1 of 2/3 *N* H_2SO_4) are placed in a centrifuge tube and 0.2 c.c. of blood or plasma are added from a pipette. The pipette is rinsed by drawing the fluid in and out, and the tube is thoroughly shaken. After centrifugation 1.0 c.c. of the supernatant fluid is re-

*Dissolve 1.853 g. of red mercuric oxide in an excess of concentrated nitric acid and dilute the solution to one liter. The solution should be checked by titrating against a standard NaCl solution (see Kolthoff and Furman, Volumetric Analysis, Vol. II, John Wiley & Sons, New York, 1929, p. 219).

moved and titrated in a small test tube, using 1.0 drop of nitroprusside solution. (Titration value – 0.02) × 1000 = mg. NaCl per 100 c.c. of blood. The authors recommend that plasma be used instead of whole blood for the chloride in blood varies with the plasma corpuscle ratio due to the fact that blood cells contain only about half as much chloride per volume as plasma.

SUMMARY

A mercurimetric method is given for the determination of chlorides in blood and plasma. By check determinations it has been found to be as accurate as the standard silver methods now in use.

The titration is made directly upon the Folin-Wu filtrate and the end point is easier to observe than that obtained with the methods now in use.

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NOTE ON THE DETERMINATION OF PROTEIN IN SERUM BY A DIRECT MICRO KJELDAHL METHOD*

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IN A previous note[1] one of the authors described a modification of the micro kjeldahl method for determining proteins in blood serum. In the present communication a further slight modification is described which has been in use in this laboratory during the last eighteen months, and which has been found to be very convenient. The method is based upon the separation of albumin and globulin by the technic of Howe,[2] oxidation of the solutions containing protein with the acid oxidizing reagent of Folin and Wu[3] and direct nesslerization of ammonia in the presence of Rochelle salt. Under the conditions described there is no precipitation of mercury salts and the colorimetric reading can be made without difficulty.

The technic used is described below.

Apparatus.—(1) Folin digestion tubes of pyrex glass, graduated at 35 c.c. and 50 c.c.; (2) Pyrex glass tubing of the smallest bore obtainable cut in 6 to 10 mm. lengths; (3) micro burner; (4) ring stand with an adjustable clamp.

Reagents.—(1) Nine-tenth per cent sodium chloride; (2) concentrated sodium sulphate, prepared as follows: dissolve 22.2 gm. of anhydrous salt (Merck's Blue Label, acid free) in 75 to 90 c.c. of hot distilled water, pour into a flask graduated

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at 100 c.c. and place in an incubator at 37°; place some distilled water in the incubator; when the temperature of the incubator is reached by both liquids, dilute the sodium sulphate to the mark with the distilled water; the solution is supersaturated at room temperature, and must be kept in the incubator; (3) 10 per cent sodium tungstate solution; (4) twelfth normal sulphuric acid; (5) Folin-Wu acid digestion mixture, prepared by pouring into 100 c.c. of distilled water an equal volume of a mixture prepared as follows: to 50 c.c. of a 5 per cent copper sulphate solution add 300 c.c. of 85 per cent phosphoric acid and mix; add 100 c.c. of concentrated sulphuric acid (free from the least trace of ammonia) mix; protect from ammonia fumes; (6) Rochelle salt, a 10 per cent solution; (7) nitrogen standard, prepared by dissolving 0.1414 gm. of the purest ammonium sulphate and making up to the mark in 100 c.c. volumetric flask with distilled water; a convenient dilute standard is made by diluting 10 c.c. of this stock standard to 100 c.c. in a volumetric flask with distilled water; (8) Nessler's solution prepared according to the directions of Folin and Wu, as follows: 150 gm. of potassium iodide and 110 gm. of iodine are placed in a 500 c.c. Florence flask; 100 c.c. of water and an excess (140 to 150 gm.) of metallic mercury are added; the flask is then shaken continuously for seven to fifteen minutes until the color of the iodine has disappeared and is replaced by the greenish color of the double iodide. The solution, which becomes quite hot, must be cooled under the tap when the color begins to get pale and before the red has wholly disappeared. Then decant, wash the mercury and flask with a large volume of water and make the supernatant fluid and washings up to two liters. The final Nessler's solution is prepared from this double iodide and from 10 per cent sodium hydroxide, which should be standardized with an accuracy of at least 5 per cent and which must be free from carbonate. To ensure the latter, prepare a strong solution of sodium hydroxide containing 55 gm. in each 100 c.c. and allow to stand for several days or weeks until the sodium carbonate has precipitated and settled; then decant and dilute to a strength of 10 per cent just before using. To prepare the final Nessler's solution mix 3500 c.c. of this 10 per cent sodium hydroxide, 750 c.c. of the double iodide solution described above, and 750 c.c. of distilled water. The alkalinity of this solution is important; 20 c.c. of normal hydrochloric acid should require between 11 and 11.5 c.c. of the finished Nessler's solution to give an endpoint with phenolphthalein. The solution is clear when first prepared; if a precipitate forms, the supernatant fluid may safely be decanted and used.

Preparation of Solutions for Analysis.—For total protein dilute 0.5 c.c. of serum accurately to 25 c.c. with 0.9 per cent sodium chloride; take 0.5 c.c. and proceed with the digestion as described below. Determine albumin after removing globulin in the following manner: treat 0.5 c.c. of the serum with 15 c.c. of the 22.2 per cent sodium sulphate solution for three to four hours. Filter in an incubator using a No. 50 Whatman filter or a paper of similar grade. To protect against loss by evaporation, the funnel should be placed directly in the mouth of the flask and the top of the funnel covered with a watch glass. If the filtrate is not perfectly clear return to the filter paper. Take 0.5 c.c. of the filtrate, which contains albumin but not globulin, and proceed with the digestion. For the nonprotein nitrogen add to 2 c.c. of the serum, 16 c.c. of twelfth normal sulphuric acid and 2 c.c. of 10 per cent sodium tungstate. Mix and filter. Take 5 c.c. of the filtrate and proceed with

the digestion. (Should the blood urea nitrogen be above normal use less of the filtrate, i. e., when the blood urea nitrogen is more than 100 mg. per 100 c.c. take only 1 c.c. of the filtrate.)

Digestion.—Into Pyrex test tubes graduated at 35 and 50 c.c., measure the different solutions prepared as described in the preceding paragraph. Add 1 c.c. of the digestion mixture and 1 piece of Pyrex tubing. Place the tube in an adjustable clamp so that the bottom of the tube is about one inch above a micro burner, and heat with a flame that just touches the tube until the water is expelled and white fumes appear. Cover the tube with a watch glass and heat until the brown color entirely vanishes. Remove flame; cool until white fumes have nearly subsided and tilt the tube to an almost horizontal position. With a curved medicine dropper, carefully place a few drops of distilled water at the mouth of the tube and let them run down the sides. When the water mixes with the digested material, some bumping occurs but nothing will be lost if the water is added cautiously. As soon as the bubbling ceases make up to a volume of 10 to 15 c.c. with distilled water. Cool to room temperature. For the standards take 3 c.c. and 5 c.c. of the dilute solution (equivalent respectively to 0.09 and 0.15 mg. nitrogen) in Pyrex tubes like those used for the unknowns, and add 1 c.c. of digestion mixture to each. Add 1 c.c. of 10 per cent Rochelle salt to the standards and unknowns. Make all tubes up to 35 c.c. with distilled water, add 15 c.c. of Nessler's solution, mix thoroughly and compare each unknown with the standard nearer to it in color.

Calculations.—

$$\text{N. P. N.} = \frac{S}{U} \times \text{St.} \times \frac{1000}{V}$$

$$\text{T. P.} = \left[\left(\frac{S}{U} \times \text{St.} \times \frac{100}{0.01} \right) - \text{N. P. N.} \right] \times \frac{6.25}{1000}$$

$$\text{Alb.} = \left[\left(\frac{S}{U} \times \text{St.} \times \frac{200}{0.0323} \right) - \text{N. P. N.} \right] \times \frac{6.25}{1000}$$

$$\text{Glob.} = \text{T. P.} - \text{Alb.}$$

Where S = reading of standard; U = reading of unknown; St. = value of standard in mg. nitrogen; T. P. = total protein; Alb. = albumin; Glob. = globulin. Total protein, albumin, and globulin are expressed as grams per 100 c.c., nonprotein nitrogen as mg. per 100 c.c. V = volume of filtrate used.

When the oxidation technic was applied to solutions containing urea and glucose the results were entirely satisfactory. For standardizing the method, further results obtained by it on a series of ten sera were compared with results given by a macro kjeldahl procedure on 3 and 5 c.c. of the same specimens. In these controls the final determination was made by titration with standard acid after the ammonia had been distilled from a sulphuric acid digestion mixture. The averages of the values of the two series differed from each other by only 0.6 per cent. A comparison of results when the micro method was carried out upon ½ c.c. and 1 c.c. of the same filtrate was also made. Oxidation was more difficult when the larger amount was used, and agreement between determinations not as close as in the figures just cited. However the average difference in ten determinations was only 1.6 per cent. and the authors think such discrepancies are not greater than should be expected

when a colorimetric method of this sort is used. The method described has been in use in this laboratory for eighteen months, and between 150 and 160 determinations have been carried through in that time. It has apparently been satisfactory within the limits expected of a routine method of this type which involves the measurement of small amounts of serum and the use of the colorimeter. The method certainly possesses a marked technical advantage over the one previously described by one of us.

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A RAPID METHOD FOR THE SIMULTANEOUS DETERMINATION OF CARBON DIOXIDE CAPACITY AND UREA NITROGEN CONTENT OF BLOOD*

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IN 1914 Van Slyke and Cullen[1] and Van Slyke and Zacharias[2] showed that urea is converted quantitatively into ammonium carbonate by the action of urease. Partos[3] and Mirkin[4] showed that it is possible to estimate exactly the carbon dioxide liberated from the converted ammonium carbonate upon treatment with an acid. In 1927 Van Slyke[5] described a method for the determination of urea in blood by gasometric measurement of the carbon dioxide liberated from the ammonium carbonate formed by the action of urease. However, that method requires a large expensive apparatus, an experienced technician, and many carefully prepared reagents. These factors inhibit its use by the average physician or by a technician in the smaller laboratory.

The method described in this paper is simple and rapid. It can best be done on a simple, inexpensive, portable apparatus described by one of the authors.[6] This apparatus can be obtained from the Emil Greiner Co., New York.

REAGENTS USED

(1) 1.13 per cent KH_2PO_4 in 0.028 Normal H_2SO_4**
(2) Urease Solution†
(3) Caprylic Alcohol

*From the Pathological Laboratories of the Jewish Hospital and Crown Heights Hospital. Received for publication, June 16, 1932.

**Phosphate Reagent: Take 11.3 gm. KH_2PO_4 and dissolve in approximately 500 c.c. distilled water. Add 2.8 c.c. of 10/N H_2SO_4. Make up to a liter with distilled water.

†Urease Solution: Make fresh for each determination. In a small test tube (4" by 3/8") dissolve contents of a No. 1 capsule of Urease Squibb in 1 c.c. of Phosphate reagent. Add 1 drop of Caprylic Alcohol. Shake well until it is finely emulsified.

DRAWING OF BLOOD SAMPLES

Puncture a vein with an ordinary intravenous needle and allow about 5 c.c. of blood to flow into a tube containing a small pinch of potassium oxalate. Cork immediately. Invert the tube 2 or 3 times and let the plasma separate by gravity. Do not centrifuge. The analysis should be done as soon as cells have settled.

PROCEDURE

Raise the apparatus to position "A" (Fig. 1) by loosening the thumbscrew. Clean the apparatus by introducing 1 c.c. of phosphate reagent into the chamber. Lower and raise the leveling cup a few times and eject the solution by opening

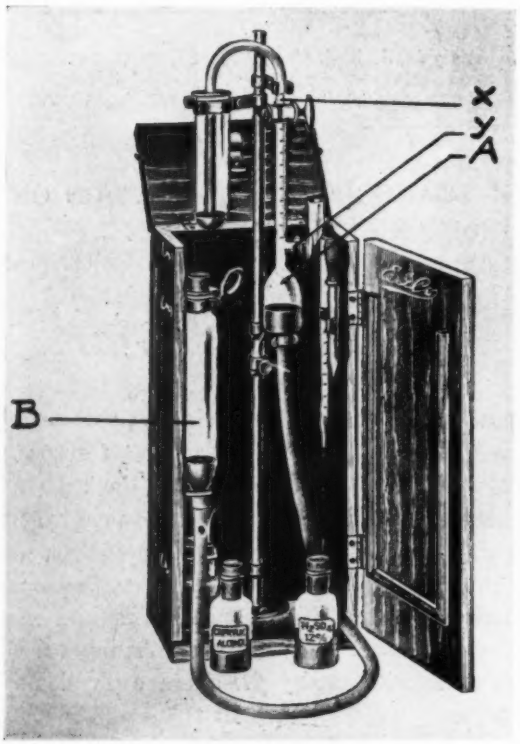

Fig. 1.

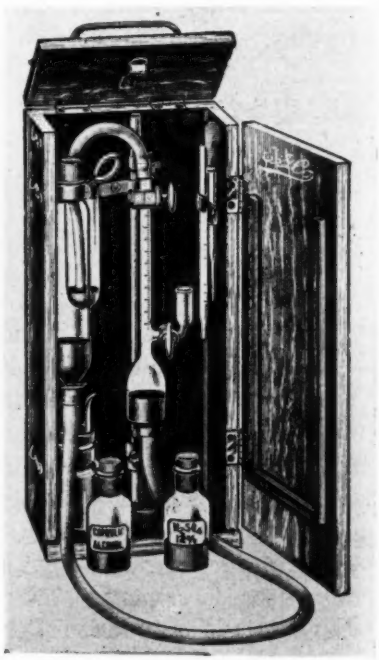

Fig. 2.

stopcock "X." Produce a vacuum by lowering the mercury leveling cup. Raise the leveling cup. If the apparatus is gas free, the mercury will strike the upper cock with a sharp click. If this click is not heard, allow the accumulated gas or fluid to escape by opening the upper cock "X," and repeat lowering and raising the mercury leveling cup until the cleck is heard. With the leveling cup in position "B" introduce 0.5 c.c. of serum or plasma into the side-arm. Add one drop of caprylic alcohol on top of the serum or plasma. Rotate the stopcock slowly until most of the fluid in the side-arm enters the chamber and displaces the mercury from above. No harm is done if the mercury column in the chamber is broken by the entrance of the serum or plasma.

Wash the cup with 0.5 c.c. of the phosphate reagent drawn up with the same pipette and allow it to enter the chamber, leaving just enough solution in the cup so that no air is possibly introduced. Seal the stopcock by running a drop of mercury into the capillary "Y." Lower and raise the mercury leveling cup a few times in order to wash down all serum or plasma adhering to the walls of the stem. Lower the leveling cup until the mercury drops to the 25 c.c. mark. Tip the box gently back and forth for one minute, thus agitating the solution in the chamber. This may also be done by tapping the sliding rod. The mercury in the leveling cup is now raised slowly so that it reaches the level of the mercury meniscus in the stem. Take R_1 and note the amount of CO_2 in 100 c.c. of blood by using Table I. Now introduce all of the prepared urease solution into the cham-

TABLE I

R_1 OR R_3	VOL. % CO_2 EQUIV. TO R_1	MG % UREA N. EQUIV. TO R_3	R_1 OR R_3	VOL. % CO_2 EQUIV. TO R_1	MG % UREA N. EQUIV. TO R_3	R_1 OR R_3	VOL. % CO_2 EQUIV. TO R_1	MG % UREA N. EQUIV. TO R_3
1	2*	3†	1	2*	3†	1	2*	3†
0.030		1.2	0.185	23.6	37.2	0.350	55.5	75.6
0.035		2.3	0.190	24.6	38.4	0.355	56.7	76.7
0.040		3.5	0.195	25.5	39.5	0.360	57.4	77.9
0.045		4.7	0.200	26.5	40.7	0.365	58.4	79.1
0.050		5.8	0.205	27.5	41.9	0.370	59.4	80.2
0.055		7.0	0.210	28.4	43.0	0.375	60.3	81.4
0.060		8.1	0.215	29.4	44.2	0.380	61.3	82.6
0.065		9.3	0.220	30.3	45.4	0.385	62.3	83.7
0.070		10.5	0.225	31.3	46.5	0.390	63.2	84.9
0.075		11.6	0.230	32.3	47.7	0.395	64.2	86.0
0.080		12.8	0.235	33.2	48.8	0.400	65.2	87.2
0.085		14.0	0.240	34.2	50.0	0.405	66.1	88.4
0.090		15.1	0.245	35.2	51.2	0.410	67.1	89.5
0.095		16.3	0.250	36.1	52.3	0.415	68.1	90.7
0.100		17.4	0.255	37.1	53.5	0.420	69.0	91.8
0.105		18.6	0.260	38.1	54.7	0.425	70.0	93.0
0.110	9.1	19.8	0.265	39.1	55.8	0.430	71.0	94.2
0.115	10.1	20.9	0.270	40.0	57.0	0.435	71.9	95.3
0.120	11.0	22.1	0.275	41.0	58.1	0.440	72.9	96.5
0.125	12.0	23.3	0.280	42.0	59.3	0.445	73.9	97.7
0.130	13.0	24.4	0.285	42.9	60.5	0.450	74.8	98.8
0.135	13.9	25.6	0.290	43.9	61.6	0.455	75.8	100.0
0.140	14.9	26.7	0.295	44.9	62.8	0.460	76.8	101.2
0.145	15.9	28.0	0.300	45.8	64.0	0.465	77.8	102.3
0.150	16.8	29.1	0.305	46.8	65.1	0.470	78.7	103.5
0.155	17.8	30.2	0.310	47.7	66.3	0.475	79.1	104.6
0.160	18.8	31.4	0.315	48.7	67.4	0.480	80.5	105.8
0.165	19.7	32.6	0.320	49.7	68.6	0.485	81.6	107.0
0.170	20.7	33.7	0.325	50.7	69.8	0.490	82.6	108.1
0.175	21.7	34.9	0.330	51.6	70.9	0.495	83.6	109.3
0.180	22.6	36.0	0.335	52.6	72.1	0.500	84.5	110.5
			0.340	53.6	73.3			
			0.345	54.5	74.4			

*The values in Column 2 are the same as those given by Van Slyke except that they have been modified to correspond to ½ the quantity of plasma or serum used, and have been corrected for CO_2 contained in the Phosphate reagent and caprylic alcohol.

†The values in Column 3 have been calculated from the following formula. At 20° C.

$$\text{Mg. \% Urea N.} = \frac{R_2 - R_1 - 0.025}{0.0043}$$

The value 0.025 is a correction for the constant volume of CO_2 liberated from blank determinations of the PO_4, urease, and caprylic alcohol reagents.

ber, leaving just enough in the cup so that no air is possibly introduced. Seal the stopcock by running a drop of mercury into capillary "Y." Lower and raise the mercury leveling cup a few times in order to bring the urease solution in contact with the plasma or serum which wets the walls of the stem. Lower the leveling cup until the mercury in the chamber is at the 25 c.c. mark. Shake for two minutes. Then raise the leveling cup to position "B." Introduce 1 c.c. of the phosphate reagent into the chamber, leaving just enough solution in the cup so that no air is possibly introduced into the chamber. Seal capillary "Y" with a drop of mercury.

Lower leveling cup so that mercury in the chamber reaches the 25 c.c. mark. Shake for one minute. Raise mercury in leveling cup slowly until it reaches the level of mercury in the chamber. Take R_2. The difference between R_1 and R_2 gives R_3. Note amount of urea nitrogen in blood corresponding to R_3 from Table I.

DISCUSSION OF TABLE I

Both the CO_2 combining power and the Urea Nitrogen content of blood can be read directly from this table. For example:

If $R_1 = 0.345$
and $R_2 = 0.435$

then $R_3 = 0.090$

The value in Column 2 corresponding to R_1 or 0.345 in Column 1 is 54.5 volumes per cent of CO_2. The value in Column 3 corresponding to R_3 or 0.090 in Column 1 is 15.1 mg. per cent of urea nitrogen.

TABLE II

COMPARISON OF CO_2 DETERMINATIONS WITH THE SIMPLIFIED APPARATUS AND THE VAN SLYKE APPARATUS

BLOOD NO.	SIMPLIFIED	VAN SLYKE	VOLUME PER CENT DIFFERENCE
1	26.5	24.2	2.3
2	40.0	38.5	1.5
3	56.7	54.8	1.9
4	40.0	39.5	0.5
5	34.2	31.9	2.3

DISCUSSION OF TABLE II

Table II shows slight differences between the two methods. Considering the original Van Slyke method as the standard, the simplified method is accurate with 1.7 volumes per cent CO_2.

TABLE III

COMPARISON OF UREA NITROGEN DETERMINATIONS DONE BY THE SIMPLIFIED METHOD AND BY THE VAN SLYKE-CULLEN[7] TITRATION METHOD

BLOOD NO.	SIMPLIFIED	VAN SLYKE-CULLEN	MG. PER CENT DIFFERENCE
1	29.1	28.5	0.6
2	25.6	26.5	0.9
3	67.4	65.0	2.4
4	14.0	15.1	1.1
5	42.6	43.9	1.3

DISCUSSION OF TABLE III

Table III shows a number of analyses done by the simplified method and the Van Slyke-Cullen Titration method. The difference in the results vary from 0.6 to 2.4 mg. with a maximum error of 7 per cent.

TABLE IV

REPRODUCIBILITY OF THE SIMPLIFIED METHOD

BLOOD NO.	VOLUMES % CO_2	AVERAGE DEVIATION	MG. % UREA N	AVERAGE DEVIATION
1	45.8	0.4	11.6	0.0
	45.8		11.6	
	44.9		11.6	
2	40.0	0.3	50.0	1.0
	39.1		50.0	
	39.1		52.3	

DISCUSSION OF TABLE IV

Table IV shows extremely consistent results with the simplified method.

DISCUSSION

The simplified apparatus is particularly suited for the CO_2 and urea nitrogen determinations because of the wide range over which it yields accurate results. The simplified CO_2-Urea method has several advantages over the ammonia estimation method for urea. It dispenses with apparatus required for the aeration or distillation of the ammonia. Apparatus for titration and colorimetric reading are not necessary. Exact standard solutions for titration or for colorimetric comparison in nesslerization are eliminated. The result is a diminution in sources and likelihood of error, and a gain in rapidity.

The phosphate reagent is of sufficient acidity to liberate all of the CO_2 (see Table II) and yet weak enough to produce optimum buffer conditions so that the action of urease is not inhibited. The urease itself being alkaline neutralizes the slight excess of phosphate reagent present and brings the P_H up to the proper level. Under these conditions the action of the urease is almost instantaneous even with uremic blood.

If the simplified method is used for the analyses of fluids other than plasma or serum, then the P_H of the sample used must be carefully adjusted.

SUMMARY

1. A simple rapid method is described for the simultaneous determination of CO_2 and urea nitrogen on 0.5 c.c. of blood.

2. The determination may be done on plasma or serum.

3. The accuracy for CO_2 determinations is within 3 volumes per cent.

4. The accuracy for urea nitrogen determinations is within 6 per cent.

The results obtained with 0.5 c.c. samples are equal in accuracy to those obtained with the usual 3 c.c. samples by the ammonia titration method. Hence only 1/6 the amount of blood is required by the simplified method.

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THE ROSE BENGAL TEST OF HEPATIC FUNCTION*

A Spectroscopic Method

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DYE elimination tests of hepatic function have proved their usefulness and are at present a part of the armamentarium of the internist. The reliability of such tests depends to a great extent upon the nature of the dye used and on the method by which its elimination through the liver is determined.

Rose Bengal† (di-iodo-tetra-chlor-fluorescein) is very suitable for this purpose because it is excreted exclusively by the liver. The technic of the test as described by Epstein, Delprat and Kerr[1] has the important advantage of eliminating artificial standards by using as a standard for comparison the concentration of rose bengal in the patient's blood two minutes after the injection of the dye. In this way the significance of all individual peculiarities of weight, blood volume, etc., with the consequent possible errors are eliminated. In addition all adults can receive the same amount of rose bengal, making calculation of dosage unnecessary.

There remain two sources of error inherent in the colorimetric reading of samples which are overcome by the spectroscopic method to be described. The first of these is the deeper yellow color of the eight minute and sixteen minute samples due to lowering of the concentration of the dye and the consequently greater thickness of the layer of plasma necessary to match the two minute specimen. This is especially noticeable in normal and borderline cases and is more pronounced in the sixteen minute sample where it often amounts to matching intensities of different colors.

Attempts to overcome this difficulty by precipitating the blood proteins with acetone were unsuccessful because a sufficiently large and inconstant

*From the Department of Medicine, University of California Medical School.
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†This dye may be obtained in ampule form, from the firm of Hynson, Westcott and Dunning, Baltimore, Maryland.

portion of rose bengal is carried down in the precipitate to distort the results of the test. The second potential source of error in the colorimetric method is the presence in the blood of increased amounts of pigment or colloidal substances, such as bilirubin in jaundice, hemoglobin due to hemolysis, and lipoids in postprandial or diabetic states.

In order to avoid these difficulties, Snapper and Spoor[2] proposed the use of the spectroscope for quantitative determinations of the dye per 100 c.c. of plasma. Their method involves the combined use of a Hellige colorimeter and a spectroscope, the readings being made on the colorimeter when the absorption band of rose bengal is just visible in the spectroscope. While a step in the right direction, this method depends on the use of artificial

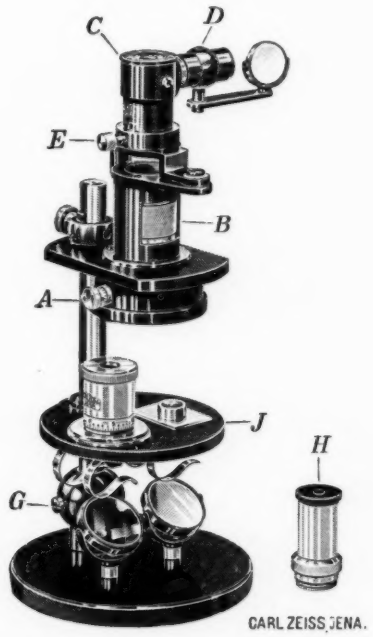

Fig. 1.—Comparison spectroscope with absorption vessel of variable depth of fluid.

standards and for accuracy requires identical conditions in regard to intensity of illumination and aperture of the spectroscope.

The method proposed here is based on the use of a comparison spectroscope (such as seen in Figs. 1 and 2) and combines the advantages of the colorimetric and spectroscopic methods.

Technic.—Ten c.c. of a 1 per cent solution of rose bengal in triple distilled water is injected into a vein of one arm. Two minutes, eight minutes, and sixteen minutes after the injection, 5 c.c. of blood is collected from a vein of the other arm into a syringe and transferred to test tubes. The syringe and test tubes are previously rinsed with a solution of potassium oxalate to prevent coagulation. The blood samples are centrifuged until the erythrocytes separate from the plasma. The latter is pipetted off and di-

luted with twice its volume of distilled water. From here on the procedure depends on the type of spectroscope used. If a comparison spectroscope similar to the one shown in Fig. 1 is available, the two minute and one of the other samples of diluted plasma are placed in the cups of the instrument. The absorption band of rose bengal appears in the green part of the spectrum (555 $\mu\mu$) and is of greater width and intensity in the two minute specimen.

By manipulation of the screw the thickness of the plasma layer in the two minute sample is reduced until the absorption bands are equal in width and intensity. Then the figures on the scale are read and the percentage of the dye in the second sample easily calculated assuming the two minute specimen to contain 100 per cent of rose bengal. The same procedure is repeated using the two minute and the remaining samples.

Fig. 2.—Hand spectroscope with test tube condenser.

If a simpler hand spectroscope shown in Fig. 2 is used the amount of diluted plasma in the test tubes must be measured. It is best to begin with the two minute (A) and the sixteen minute (C) samples, and by gradually adding measured amounts of water to Tube A, effect a match of the two absorption bands. The concentration of the dye in Tube C is calculated according to the following formula: $X = \frac{A \times 100}{A + A_1}$; where A is the amount of plasma in the first specimen, and A_1, the amount of water added to Tube A. Following this the eight minute (B) sample is diluted in the same manner to match the sixteen minute (C) sample, and the concentration of rose bengal in Tube B is obtained from the following equation: $Y = \frac{X\ (B + B_1)}{B}$, where B is the amount of plasma in the second specimen and B_1 the amount of water added to Tube B.

With either type of spectroscope, no precautions need to be observed regarding the intensity of illumination or aperture of the instrument.

The figures thus obtained indicate the amount of rose bengal still present in the circulating blood eight and sixteen minutes after the injection of the dye.

In order to ascertain the practical value of the spectroscopic method of reading the rose bengal liver function test, both the spectroscopic* and the colorimetric methods were carried out on identical blood samples in 100 instances.

RESULTS

1. *Normal Cases.*—In 37 patients with normal elimination of rose bengal, the average of spectroscopic readings for both samples was 6.8 per cent higher than that of the corresponding readings with the colorimeter. The significance of this difference is that the upper limit of normal which has been accepted for the colorimetric method as 50 per cent of the standard at the end of eight minutes, and 30 per cent at the end of sixteen minutes, must be changed to 55 per cent and 35 per cent respectively if the spectroscopic method is used. The explanation of this difference probably is that the yellowish color of the plasma obscures the dye to a greater extent in the last two specimens.

2. *Cases With Hepatic Damage.*—In 42 individuals with abnormal retention of rose bengal, the average of all spectroscopic readings was 5.6 per cent higher than that of the colorimetric readings, thus confirming the quantitative differences observed in 37 normal cases.

3. *Interfering Substances in the Plasma.*—a. Jaundice: 16 rose bengal tests were performed on 13 patients with clinical jaundice having an icterus index from 15 to 105. Of these it was impossible to carry out the colorimetric determination on 13 eight minute samples and on 14 sixteen minute samples. On the other hand the presence of bilirubin in the plasma did not hamper the spectroscopic determinations of rose bengal in any of the samples.

As a crucial test the plasma of the eight and sixteen minute specimens from a case of jaundice with an icterus index of 81 were diluted with the icteric plasma of the same patient instead of water and still the spectroscopic readings could be carried out with ease and were equal to those obtained in the usual manner. In a patient with hemolytic jaundice having an icterus index of 30 the colorimetric readings were modified by the icterus to show abnormal retention of rose bengal whereas the spectroscopic determinations revealed normal excretion of the dye.

b. Hemolysis: On four occasions considerable hemolysis was visible in the centrifuged plasma. In all cases the colorimetric reading was either impossible or much higher than the spectroscopic one. In the spectroscope the presence of hemoglobin is manifested by two absorption bands (540 $\mu\mu$ and 580 $\mu\mu$), one on each side of the rose bengal band. All four specimens could be read satisfactorily. However, when very marked artificial hemolysis is

*The hand spectroscope shown in Fig. 2 was used for this work. Since then the instrument shown in Fig. 1 has been employed in 150 cases with excellent results.

produced the hemoglobin bands encroach on the rose bengal band and prevent even the spectroscopic determination.

c. Lipemia: In one case of marked cholesteremia (1075 mg. of cholesterol per 100 c.c. of blood), the colorimetric reading of the eight minute sample was abnormally high whereas the sixteen minute sample could not be matched satisfactorily. There were no difficulties in making determinations with the spectroscope on either sample. Hence it is no longer necessary with this method to delay the rose bengal test for four hours after a meal.

Summary.—A spectroscopic method of reading the rose bengal liver function test is described. This method retains all advantages of the colorimetric method and renders the test more reliable. In addition it permits the test to be carried out in spite of jaundice, moderate hemolysis and lipemia.

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SUPERSATURATION OF ANTIGENIC BEEF-HEART EXTRACTS WITH CHOLESTEROL AND ITS EFFECT ON THE SENSITIVITY AND SPECIFICITY OF THE COMPLEMENT FIXATION REACTION*

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PORGES, Neubauer and their collaborators[1, 2] showed that as the percentage of cholesterol in relation to lecithol in solution increases, the precipitation of this complex by human serum, and especially by positive syphilitic serum, increases abruptly to a maximum. Further increase in the cholesterol content leads to a gradual reduction in the precipitation. In a previous publication I stated that the same held true with regard to the complement fixation reaction.[3] The following experimental series were designed for the purpose of investigating this phase of the complement fixation reaction:

Dry beef-heart powder was rendered free from ether-soluble substances. It was extracted with 95 per cent grain alcohol for five days at room temperature. This was labeled Extract II. The same beef-heart powder was again extracted with hot grain alcohol, and the material labeled Extract I. Two hundred and fifty cubic centimeters of Extract I were evaporated to dryness and the residue redissolved in 200 c.c. of Extract II. This was labeled Extract III.

To portions of these extracts cholesterol was added as indicated in Table I. Antigen titrations were carried out as per Kolmer.[4] Final values were obtained by averaging the dilution at which a very faint tint of red appeared (denoted in the tables as V. F. T. R.) and the dilutions at which there appeared a barely observable increase in the red tint. The results are recorded in Tables I, II and III.

*From the Clinical Laboratory of the Public Health Institute.
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TABLE I

EXTRACT I

% CHOLESTEROL	V.F.T.R. IN DILUTION	SLT. INCREASE, IN DILUTION	AVERAGE DILUTION
0.00	1-1200	1-1800	1-1500
0.25	1-3600	1-3800	1-3700
0.50	1-5000	1-5400	1-5200
1.00	1-4800	1-5200	1-5000
2.00	1-4200	1-4600	1-4400

TABLE II

EXTRACT II

% CHOLESTEROL	V.F.T.R. IN DILUTION	SLT. INCREASE, IN DILUTION	AVERAGE DILUTION
0.00	1-2800	1- 4800	1-3800
0.60	1-4400	1- 5200	1-4800
0.80	1-4800	1- 5600	1-5200
1.00	1-5600	1- 7200	1-6400
1.50	1-7200	1- 9600	1-8400
2.00	1-8000	1-10800	1-9400
2.50	1-8200	1-10800	1-9500

TABLE III

EXTRACT III

% CHOLESTEROL	V.F.T.R. IN DILUTION	SLT. INCREASE, IN DILUTION	AVERAGE DILUTION
0.40	1- 5200	1- 6200	1- 5700
0.60	1- 7000	1- 8000	1- 7500
0.80	1- 8400	1- 9000	1- 8700
1.00	1- 9100	1-11200	1-10150
1.50	1-10400	1-12400	1-11400
2.00	1-10800	1-13500	1-12150
2.50	1-10000	1-12000	1-11000
3.00	1-10000	1-12000	1-11000
3.50	1- 8000	1-10000	1- 9000

The tables show that in complement fixation as in antigen precipitation the increase in the antigenic properties of the reagent with the increase in the percentage of cholesterol reaches a maximum which may or may not be the optimum. Beyond that maximum the antigenic power of the reagent becomes inversely proportional to the cholesterol content.

In the instance of Extract I the maximum was attained with 0.5 per cent cholesterol; in the case of Extract II, with 2.5 per cent cholesterol, and in the case of Extract III, with 2 per cent cholesterol. Evidently the percentage of cholesterol required to bring the beef-heart extract to its maximum reactivity depends upon the lipid concentration of the extract; beyond a certain lipid concentration the percentage of cholesterol required to manifest the maximum reactivity of the antigenic extract also begins to decline. This is in accord with the general principles of colloidal reactions, and follows the so-called "colloidal type-curve" discussed in another paper.[5]

The data recorded in Tables I, II, and III likewise show that a reagent having any desired antigenic titer can be obtained for the complement fixation reaction by

manipulating the lipid concentration of the alcoholic extract and its ratio to the cholesterol content. Eagle[6] prepared an alcoholic extract containing 0.6 per cent cholesterol and 0.6 per cent sitosterol (corn germ sterol). This reagent gave an antigenic titer as high as 1-10000 dilution. Using an especially strong positive serum and Extract II (normal alcoholic beef-heart extract) fortified with 1.25 per cent cholesterol, I obtained a reagent which gave an antigenic titer as high as 1-10200 dilution. It is not necessary, therefore, to complicate the reaction by the use of more than one type of sterol to obtain a reagent possessing an antigenic titer as high as 1-10000 or higher.

All serologic laboratory reactions based on alcoholic beef-heart extract, fortified with sterol, as the antigenic reagent, are basically immunologic reactions. However, they are specific for syphilis only when kept at an experimentally established level of sensitivity. The sensitivity of a fortified beef-heart extract in complement fixation is determined by its lipid content and the percentage of sterol added, while the proper balance between sensitivity and diagnostic specificity is attained by establishing the proper ratio between the beef-heart lipid and the fortifying sterol. This ratio varies with each extract. The chemistry of the lipid in particular and of the fixation reaction in general is still insufficiently known. A direct and scientific method for the standardization of the antigenic reagent is therefore yet to be developed. At the present time the correspondence between the laboratory results and the clinical findings of a large number of cases remains the only rational guide in judging the suitability of an antigenic reagent for routine laboratory purposes; and since the reaction per se is nonspecific, it cannot be assumed a priori in every case that the reagent possessing the highest titer must prove the most sensitive and at the same time the more specific.

TABLE IV

EXTRACT I

SPEC. NO.	KOLM.	KAHN	PERCENTAGE OF CHOLESTEROL AND DILUTION			
			NON-CHOL. 1-200	0.5% CHOL. 1-550	1.0% CHOL. 1-480	2.0% CHOL. 1-440
1	0.0	0.0	0	0	2	Tr
2	0.0	0.0	0	0	1	0
3	0.0	0.0	0	2	3	2
4	0.0	0.0	0	0	0	4
5	0.0	0.0	0	0	0	4
6	0.0	0.0	0	0	3	0
7	0.0	0.0	0	1	4	4
8	0.0	0.0	0	1	3	1
9	0.0	0.0	P-M*	4	4	4
10	0.0	0.0	0	0	1	0
11	3.2	0.Tr	0	0	0	0
12	0.0	0.0	0	2	3	3
13	0.0	0.0	0	0	2	0
14	0.0	0.0	0	Tr	2	0
15	0.0	0.0	0	0	2	0

*P-M = Plus-Minus; Numerals 1, 2, 3, 4 indicate degrees of complement fixation; Tr = trace; 0 = negative for fixation.

TABLE V

EXTRACT II

SPEC. NO.	KOLMER	KAHN	PERCENTAGE OF CHOLESTEROL AND DILUTION					
			0.4% 1-380	0.6% 1-480	0.8% 1-520	1.0% 1-640	1.5% 1-840	2.0% 1-940
1	0.0	0.0	Tr	Tr	1	1	3	3
2	2.2	0.0	3	3	3	3	3	4
3	0.0	Tr.Tr.	0	0	0	0	1	4
4	0.0	0.0	0	0.	0	0	1	3
5	4.Tr	0.0	4	4	4	4	4	4
6	0.0	0.0	Tr	2	3	3	4	4
7	0.0	0.0	Tr	2	3	3	4	4
8	0.0	1.1	0	0	0	0	3	4
9	0.0	0.0	0	0	0	0	1	1
10	0.0	0.0	0	0	0	0	1	3
11	0.0	0.0	0	0	0	0	0	1
12	0.0	0.0	0	0	0	0	0	1
13	0.0	0.0	0	0	0	0	0	1
14	0.0	0.0	0	0	0	0	1	1

TABLE VI

SP. NO.	KOLM.	KAHN	PERCENTAGE OF CHOLESTEROL AND DILUTION								
			0.4% 1-670	0.6% 1-750	0.8% 1-870	1.0% 1-1000	1.5% 1-1150	2.0% 1-1200	2.5% 1-1100	3.0% 1-1100	3.5% 1-900
1	0.0	0.0	0	0	0	0	1	1	1	Tr	0
2	0.Tr	0.1	1	3	3	4	4	4	4	4	4
3	0.0	0.0	0	0	0	0	Tr	Tr	2	1	Tr
4	0.0	0.0	0	0	0	0	1	1	1	1	0
5	2.2	4.4	0	2	3	3	4	4	4	4	4
6	0.0	0.0	0	0	Tr	1	3	3	3	3	3
7	0.0	0.0	0	0	0	0	0	0	0	1	0
8	0.0	0.0	0	0	0	0	0	0	0	1	0
9	0.0	0.0	0	0	0	0	0	0	0	1	0
10	0.0	0.0	0	0	0	0	Tr	0	1	1	Tr
11	0.0	0.0	0	0	0	0	0	0	0	1	0
12	0.0	0.0	0	0	2	3	4	2	2	1	1
13	0.0	0.0	0	0	0	0	1	Tr	Tr	1	Tr
14	0.0	0.0	0	0	Tr	1	1	2	4	4	3
15	0.0	0.0	0	0	0	0	0	0	1	0	0
16	0.0	0.0	0	0	Tr	Tr	1	2	3	4	4
17	0.0	0.0	0	0	0	Tr	1	2	4	4	3
18	0.0	0.0	0	1	3	4	4	4	4	4	4
19	0.0	0.0	0	0	0	0	0	0	0	1	0
20	0.0	0.0	0	0	0	0	0	0	0	1	0
21	0.0	0.0	0	0	1	1	2	3	3	3	3
22	0.0	0.0	0	0	0	0	0	4	0	0	0
23	0.0	0.0	0	0	0	0	Tr	4	1	Tr	0
24	0.0	0.0	0	0	0	0	0	3	0	0	0
25	0.0	0.0	0	0	0	0	0	4	0	0	0
26	0.0	1.2	0	1	3	3	4	4	4	4	4
27	0.0	0.0	0	0	0	0	0	4	0	0	0
28	0.0	0.0	0	0	0	0	0	4	0	0	0
29	1.1	0.0	0	3	3	4	4	4	4	4	4
30	0.0	0.0	0	0	3	3	4	4	4	4	4

The following experiments support these views:

Fifty-five blood-serum specimens were tested by the antigens prepared from Extract I; 72 specimens were tested by the antigens prepared from Extracts II and III. The dilutions of the antigens used represented ten units of the corresponding titers. Of the 55 specimens tested by the antigens prepared from Extract I, 31 were negative and 9 were positive by the experimental procedure and by the standard Kolmer[4] and by the two-tube Kahn test,[7] which were performed in all cases for control purposes. The results of the remaining 15 specimens were recorded in Table IV. Forty-seven of the specimens tested by the antigens prepared from Extract II were negative and 10 were positive by the experimental and standard routine procedures. The results obtained with the remaining 14 specimens are recorded in Table V. Thirty-three of the specimens tested by the antigens prepared from Extract III were negative and 9 were positive by all the tests employed. The results of the remaining 30 specimens are recorded in Table VI.

A brief summary of the histories of the cases, the results of which are recorded in Table VI, follows:

CASE 5.—Syphilis latent (1928), treated. Clinical examination, spinal fluid, and routine serologic tests negative.

CASE 6.—Had syphilitic infection twenty-five years ago. Reported to the Institute for a check-up examination. Routine serologic tests negative.

CASE 9.—Kahn test occasionally gives a plus-minus reaction, while the Kolmer test is consistently negative. Patient is under observation for syphilis.

CASE 18.—Neurosyphilis. Routine serologic tests are consistently negative.

CASE 21.—Neurosyphilis. Routine serologic tests at first were weakly positive. Following treatment they became persistently negative.

CASE 26.—Cardiovascular syphilis. Routine serologic tests are inconsistent and weak, with the Kolmer predominantly negative.

CASE 27.—Syphilis secondary, recurrent. Routine serologic tests, at first positive, became consistently negative following treatment.

CASE 29.—General paresis. Routine serologic tests inconstant. On some occasions all routine and special tests gave negative results.

CASE 30.—Central nervous system syphilis, asymptomatic, late. Kolmer test towards end of treatment became consistently negative, and the Kahn test giving inconstant weakly positive results. Finally all tests became consistently negative.

Of the remaining 29 cases none had any clinical or routine serologic findings suspicious of syphilis. With a few exceptions these cases were under treatment for gonococcus infection.

DISCUSSION

By increasing the lipid and cholesterol content of the antigen, its titer and sensitivity can be raised so that reactions will result from serum specimens of nearly all patients having had syphilis. The following considerations tend to indicate that such a procedure can hardly be approved at the present.

1. As stated in one of the preceding paragraphs, the complement fixation reaction is specific only within clinically established limits. If its sensitivity is raised much above the level of the presently recognized routine tests, the incidence of nonspecific reactions will increase considerably.

2. The transition from primary to the early and late secondary stages of syphilis is associated with definite changes in the intensity of the serologic reaction.[8]

3. The reduction in the degree of positivity of blood tests is regarded as an encouraging index of efficient therapy.

4. Repeated negative serologic results are important criteria of cure when clinical findings also indicate that the progress of the disease has been arrested.

If through the introduction of supersensitive antigens all serums from syphilitic individuals, regardless of the amount of treatment received, should be made to yield strongly positive results, then new criteria for judging the progress of the infection and of the effectiveness and sufficiency of treatment would have to be evolved.

The Kolmer complement fixation and the Kahn antigen precipitation tests have been in my experience thoroughly trustworthy for routine purposes. The technical details are well defined in each; the level of reactivity of each strikes a practical medium proved by years of extensive experience; they are supplementary to each other. Nevertheless, I am not of the opinion that the combination of the two tests offers an all-sufficient and infallible system of serologic study. In isolated instances special tests should be employed. However, when evaluating the results of tests of high sensitivity the clinician must not forget that the incidence of nonspecific positives increases in direct proportion to the increase in the sensitivity of the test.

Special tests should be based on as near the same principles and general technical steps as are the adopted standard routine tests. The introduction of new fortifiers, such as corn germ sterol, leads the busy clinician to new confusion. Cholesterol alone accomplishes the same degree of sensitizing for the fixation reaction as the mixture of the two sterols. It appears logical to me that for the sake of simplicity and technical uniformity cholesterol continue to be used as the exclusive fortifying sterol in the preparation of the antigenic reagent for use in laboratory diagnosis of syphilis by complement fixation.

SUMMARY

The increase in the antigenic properties of alcoholic beef-heart extract with the increase in the cholesterol content reaches a maximum for complement fixation. This maximum depends upon the lipid concentration of the alcoholic solution. Beyond the maximum fortification the antigenic power of the reagent becomes inversely proportional to the cholesterol added.

Generally, the higher the titer of the fortified beef-heart extract, the greater is its sensitivity. When the full antigenic dose of the fortified reagent is used its sensitivity can be manipulated, on the basis of the principles discussed above, so that strongly positive reactions are obtained with serum specimens from nearly all individuals who have had syphilis, regardless of the age of the infection and of the intensity of treatment received. Such a high level of sensitivity results in the appearance of strongly positive reactions, basically of an immunologic character, but considered "false positives" from the viewpoint of the syphilologist.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TISSUE: A Device for Mounting Anatomical Preparations, Schoenburn, H. W., and Hickman, C. P. Science **77:** 287, 1933.

A plate is made by melting hard paraffin and pouring it into a form (as a box top, etc.) where it hardens to form a plate from one-fourth to one-half inches in thickness. During the process of melting, enough lampblack is added to give the mixture a deep black color, which is, of course, an advantage where a dark background is desired. To prevent curling of plates after immersion in preservative, two glass rods are embedded parallel to each other, one on each side of the plate, just before the process of hardening is begun. These rods may be of various diameters, but those from 5 to 8 mm. in diameter are quite satisfactory. The length of the rods will depend upon the length of the plate as they are placed lengthwise within the plate. Before the rods are dropped in the melted paraffin a loop or two of heavy twine is wrapped around each end of each rod so that the rods will be suspended near the middle of the plate after it hardens. Care must be taken to smear the inside of the box form with glycerine so that the hardened plate can be removed easily from the form.

The addition of some beeswax and resin to the paraffin lampblack mixture will increase to a considerable extent the rigidity of the plate, although these constituents are not absolutely necessary.

Before the plate has hardened, and is still plastic, the anatomical specimen is pressed down slightly into the soft plate to hold it in position and is fastened there with thread looped around the parts of the specimen, passed through holes, and tied at the back of the plate. Necessary labels are now attached.

When completed, the preparation is immersed in a preservative in a museum jar. Such preparations will last for an indefinite time.

ENDOSPORES, Simplified Method of Staining, Schaeffer, A. B., and Fulton McD. Science **77:** 194, 1933.

Stains.—

1. Five per cent aqueous malachite green. Dissolve, allow to stand thirty minutes; filter.

2. 0.5 aqueous safranin.

Method.—

1. Prepare films and fix as usual.

2. Flood with malachite green and heat to steaming three or four times within one minute.

3. Wash under tap water thirty seconds.

4. Apply safranin stain thirty seconds.

5. Wash, blot dry, and examine.

Spores green; cells red.

TISSUE: Improving the Staining Action of Iron Hematoxylin, Hance, R. T. Science **77:** 287, 1933.

Stock Solution.—Hematoxylin 10 per cent in 95 per cent alcohol.

Staining Solution.—Five c.c. of stock solution to 100 c.c. of distilled water. Add as much sodium bicarbonate as can be lifted on the point of a scalpel.

The neutralized stain gives excellent differentiation.

THYROID DISEASE: Blood Cholesterol In, Hurxthal, L. M. Arch. Int. Med. **51:** 22, 1933.

The blood cholesterol during fasting was determined as a routine in 505 cases of thyroid disease, along with the pulse, the weight, and the basal metabolic rate.

The lowest average values for blood cholesterol are found in patients in or near thyroid crises.

Auricular fibrillation in toxic goiter is associated with the next lowest average level of cholesterol.

The average cholesterol value in all types of exophthalmic goiter is lower than in toxic adenomatous goiter.

Recurrent hyperthyroidism is associated with cholesterol values that are almost as low as those in exophthalmic goiter.

The average cholesterol value in nontoxic goiter is normal, although the scatter is wider than expected. Age appears to raise the blood cholesterol in these cases.

Chronic thyroiditis is associated with higher average values than in any other thyroid disease except myxedema.

The level of the blood cholesterol and the basal metabolic rate bear a reciprocal relationship when judged by average values.

ARTHRITIS, Rheumatoid, Cecil, R. L. J. A. M. A. **100:** 1220, 1933.

The modern method of approach to the problem of chronic arthritis is based on the following considerations:

The majority of cases of chronic arthritis fall into one or the other of two great groups. Rheumatoid arthritis is a clearly defined clinical entity which in most cases can be accurately differentiated from osteoarthritis and from the so-called specific forms of infectious arthritis. Rheumatoid arthritis has its own pathology and usually runs a characteristic clinical course. Hypertrophic arthritis, on the other hand, appears to be a degenerative, senescent process.

Evidence is rapidly accumulating that rheumatoid arthritis (like rheumatic fever) is a chronic infection. This evidence comes from pathologic, bacteriologic, serologic, and clinical sources. Allergy probably exerts an influence on the disease, but no more than in other chronic infections.

In rheumatoid arthritis a primary focus of infection is the portal of entry for the exciting agent. In respect to its pathogenesis, the disease is analogous to gonococcal arthritis, which it often resembles closely. The foci and the infectious agents are different, the mechanism of infection the same.

By employing new bacteriologic methods, a number of investigators have succeeded in cultivating streptococci from the blood and joints of patients with rheumatoid arthritis. While these observations have not been confirmed in some laboratories, the presence of specific streptococcic agglutinins in the serum of rheumatoid patients would seem to refute the theory that these microorganisms are contaminations. Furthermore, the ease with which an experimental arthritis can be produced in rabbits by intravenous injection of these streptococci lends strong support to the proposition that rheumatoid arthritis is a streptococcic infection.

The specific streptococcic agglutinins which are found in the serums of a high percentage of patients with rheumatoid arthritis, are of considerable value in differentiating rheumatoid arthritis from other forms of chronic arthritis. The sedimentation rate is useful in separating rheumatoid arthritis and the other infectious forms from noninfectious ailments of the joints.

By making use of the agglutination and sedimentation tests and the Schilling count, the physician is now in a position not only to make a more accurate classification of arthritic patients but also, by repeating the tests from time to time, to gage the activity of the disease and the efficacy of treatment.

The rational treatment of rheumatoid arthritis embraces removal of foci of infection, emphasis on rest, and a general building-up program, consisting of carefully regulated diet, vitamins, iron and arsenic, and adequate elimination through the intestine, bladder, and skin. Streptococcus vaccine, administered intravenously, is of considerable value in many cases, though its modus operandi is not yet understood. Physical therapy, if carefully supervised, is useful in stimulating the local and general circulation. In advanced cases, orthopedic surgery may solve an otherwise baffling problem.

TISSUE: A New Paraffin Embedding Mixture, Hance, R. T. Science **77:** 353, 1933.

Difficulty in obtaining a paraffin of satisfactory consistency in which to embed and section biologic material to the development of the following formula.

A stock solution of crude rubber in paraffin is first made. Crude rubber is available in thin sheets, either smoked or unsmoked. Both kinds work equally well. The sheets of crude rubber are chopped up with a scissors and dropped in melted Parawax or any similar common paraffin. The paraffin should be smoking hot and the mixture should be stirred occasionally. Three to four hours are required to completely melt the rubber. About 20 gm. of rubber can be dissolved in 100 gm. of paraffin.

Embedding Mixture

Parawax	100 gm.
Rubber-paraffin mixture	4-5 gm.
Beeswax	1 gm.

Filter through paper (paper towels serve this purpose excellently).

This mixture is pale yellow in color, does not crystallize readily, and is of a waxy consistency that sections unusually well.

TISSUE: Microtechnical Demonstration of Insoluble Lime Salts in Tissues, Gomari, G. Am. J. Path. **9:** 253, 1933.

1. Cut or saw thin blocks of fresh tissue 1 to 2 mm. thick.
2. Fix in 80 to 96 per cent alcohol, or boil in one of the solutions mentioned. After this wash blocks in distilled water for three to four hours.
3. Impregnate in 1.5 per cent silver nitrate solution for six to ten days. Change silver solution once or twice.
4. Wash for three to four days in distilled water changed daily four to five times, until the last washing water decanted does not show the slightest turbidity when mixed with hydrochloric acid.
5. Reduce in a 5 per cent solution of sodium hypophosphite. Before use add 4 to 5 drops of a 0.1 normal sodium hydroxide solution to each 100 c.c. of reducer. Keep blocks in reducer for four to eight days.
6. Wash in running water for three to four hours.
7. Fix in a 3 to 5 per cent solution of sodium thiosulphite for two days.
8. Wash in running water for at least twenty-four hours.
9. Decalcify in a 6 to 8 per cent solution of sulphosalicylic acid.
10. Wash, embed, and so on.

Steps 3 to 7 inclusive are to be performed in the dark.

RENAL FUNCTION, In Arterial Hypertension, Ellis, L. B., and Weiss, S. J. A. M. A. **100:** 875, 1933.

In 24 cases of arterial hypertension without clinical signs of cardiac or renal failure, and in eight cases of glomerulonephritis, three types of tests of renal function were used: the usual urinalysis, urea and creatinine clearance tests, and concentration dilution tests.

In 10 of the cases of hypertension the urea clearance test gave normal results; in 9 there was a slight reduction, and in 5 it was markedly lowered.

Creatinine clearance tests, in 18 of the same cases, was normal in 13 instances, slightly reduced in 2, and markedly reduced in 3. In general, the outcome of the urea and creatinine tests tended to give parallel results.

In 22 cases in which the concentration test was performed in the patients with hypertension, the maximum specific gravity of the urine was above 1.025 in six instances; 10 times it fell between 1.020 and 1.025; and in the remaining 6 cases it fell below 1.020.

In only 2 cases did the concentration test show a definite lowering without a marked reduction in the urea or creatinine clearance tests.

For practical purposes, in most cases a carefully conducted concentration test is as sensitive an index of renal impairment as the urea and creatinine clearance tests.

Marked albuminuria or hematuria was uncommon in hypertension unless great limitation of function was detectable by other tests.

The results of this study have been analyzed in accordance with the filtration reabsorption theory of renal physiology and have been correlated as far as possible with the histologic changes in the kidney, known to occur in hypertension.

There was a trend for the degree of impairment of renal reserve to parallel the height of the arterial blood pressure, particularly the diastolic pressure. There was no correlation to be made between the degree of renal damage and the age of the patients, the symptoms or the known duration of the disease.

SPORES, A Simplified Method of Staining, Shaeffer, A. B., and Fulton, M. Science 77: 194, 1933.

Films of bacteria, made in the usual manner, are fixed by flaming three times. Flood with 5 per cent aqueous malachite green and heat to steaming 3 or 4 times within one-half minute. Wash off the excess stain under the tap for about one-half minute. Apply a 0.5 per cent aqueous safranin solution for one-half minute. Wash, blot, dry, and examine. Spores are stained green, cells red. There is no blending of the colors.

MALIGNANCY, A Quantitative Modification of the Bendien Reaction in Serodiagnosis of, Lowe, E. C. Brit. M. J. 3376: 407, 1933.

With the technic described below, which is stated to have received Dr. Bendien's approval, the author reports 95 per cent diagnostic accuracy if the clinical condition is not taken into account and 98 per cent if it is.

His conclusions are as follows from a study of 600 tests:

The primary tube reaction alone is shown to be insufficient for the diagnosis of malignancy. The triple test must be employed, and will then differentiate malignant from nonmalignant conditions in at least 95 per cent of cases.

The clinical malignancy and the degree of positiveness shown by the test closely correspond.

Postoperative cases of cancer, or those having radium, x-ray, or other forms of treatment, may be "followed up" by means of this test, and clinical information may thereby be obtained regarding prognosis and treatment.

Cases are referred to in which satisfactory clinical improvement and apparent cure is associated with the continued presence of a normal or nonmalignant serum reaction.

In some cases the serum reaction has foretold a recurrence months before clinical observations have confirmed it.

Among normal and clinical nonmalignant conditions a certain percentage of abnormal reactions occur, suggesting the possibility of recognizing in such cases a precancerous tendency.

The test is delicate and reliable, more satisfactory as a laboratory technic than any other at present available, and should be of value as a clinical aid to diagnosis and prognosis of malignancy, especially in early cases.

The technic follows:

The Reagents.—The first eight mixtures of N/10 acetic acid and N/10 ortho-sodium vanadate are prepared according to Bendien's detailed description.

The Serum.—Ten c.c. of blood is required, obtained from the patient in a fasting condition. The serum should not be hemolyzed; after standing it is centrifuged clear of cells. Fine's suggestion of using only 0.1 c.c. of serum in each reaction tube economizes serum.

The tubes must be standardized; 75 mm. in length and 7 to 8 mm. internal diameter. Gross variations cause considerable alterations in reaction.

Delivery of the small quantities of serum and reagents employed demands absolute accuracy.

For the modified test now to be described each serum is divided into three portions and marked accordingly. Approximately 1 c.c. is required for each part.

"A" is the portion to be used unheated. It is carefully diluted with an equal quantity of distilled water, well mixed, and 0.2 c.c. of the mixture is delivered into each of eight tubes; the respective reagents are added; Reagent 1 to the first tube, Reagent 2 to the second, etc.; the mixtures well shaken, and put aside to settle for sixteen to twenty-four hours. "B" is the portion to be inactivated by heating in a water-bath for half an hour at 56°. "C" is the portion in which the lipoid-protein complex is to be dissociated by ether. For this purpose at least half as much ether is added as there is serum, the whole being thoroughly shaken in a well-corked tube and set aside to separate out. This will occur as a layer above the cleared serum.

The following day the "A" (unheated serum) tubes are examined. The reactions vary from nil in which the contents of the tube are quite clear, to a complete precipitation of all protein contents of the serum, which is shown by a heavy deposit lying below a water-clear, supernatant fluid. Between these two extremes every variety of turbidity and less or more precipitation may be found. Two rows of tubes are now placed in the rack behind the "A" series. Into the first of these rows 0.2 c.c. of diluted "B" (heated) serum is placed, and into the second row 0.2 c.c. of diluted "C" (ether-treated) serum, which has been carefully pipetted from below the ether layer and then diluted. To each tube 1 c.c. of the respective reagents is added, all being shaken and allowed to stand, as in the case of the "A" series. From each serum a series of the "A," "B" and "C" series may differ markedly in certain cases, according to the amount of thermolabile substance inactivated by heating and to the dissociation effect produced by ether on the serum contents.

ESTIMATION OF DEPOSIT

The visual readings of the deposits, although they show a move to the right of varying amount in the "B" (heated serum) and a move to the left, most marked in malignancy, in the "C" (ether-treated serum) reaction, are not sufficiently accurate for quantitative estimation, and the following method is therefore employed. The tubes are centrifugalized at about 1,000 revolutions per minute and the supernatant fluid carefully removed without disturbing the deposit. The deposits are dissolved by adding, to each, 2 c.c. of N/100 NaOH, and the resulting solutions vary in concentration according to the amount of precipitate dissolved in each. By means of the Lowe interferometer (1910), as made by Zeiss, these concentrations are read in units, using a 5 mm. observation chamber, and by experimental estimation it is found each unit corresponds to 0.025 mg. A reading, therefore, of 40 units equals 1 mg. of precipitate, and this amount obtained from 0.1 c.c. of serum equals a 1 per cent precipitation. Experimentally this amount of deposit is found to be that which usually appears visually as the "first precipitation," and it was therefore arbitrarily taken that a quantitative estimation of 40 units (or nearest above) should be regarded as the "first precipitation" and the reaction classified by the reagent number in which this amount of deposit is first found.

INTERPRETATION OF FINDINGS

The tube of the "A" series in any case which gives the first reaction having been determined, the unit measure thus obtained is taken as the basal figure for the computation of that serum, and the corresponding "B" and "C" deposits are similarly estimated, with the result that from these values in interferometer units a fraction may be worked out in which the numerator is calculated by subtracting the unit value of "A" from "C" and the denominator by subtracting "B" from "A."

Reagent	6	A	B	C	6	A	B	C
Interferometer units of deposit		40	20	45		59	57	109
Fraction $\frac{C - A}{A - B}$ =		5/20				50/2		
		= Nonmalignant				= Malignant		

In cases where the thermolabile effect is marked and the increase of precipitation after ether treatment is slight (as is found in normal or nonmalignant inflammatory conditions) the fraction is less than unity. Conversely, when the thermolabile effect is slight or absent, and the precipitation after ether treatment increased or high (as is found in malignancy), the resulting fraction is equally consistently greater than unity, and this differentiation holds good irrespective of whether the first "A" reaction occurs in the lower or in the higher numbered tubes. Normal serums, as a rule, show a first precipitation with Reagent 6. Nonmalignant infective and malignant conditions may show a first reaction in any of the series according to the severity of the pathologic condition.

TUBERCULOSIS: Tuberculin and the Schilling Differential Blood Count in the Diagnosis and Classification of Tuberculosis, Bredeck, J. F. Am. Rev. Tuber. **27:** 377, 1933.

From a study of the two reactions in a series of 305 cases, the following conclusions are drawn:

1. The interpretation of the clinical importance of the subcutaneous tuberculin test can best be judged by the Schilling blood differential count.

2. The Schilling count is a more accurate measure of induced focal reactions than are fever, general tuberculin reactions, physical signs, or x-ray.

3. A classification of the various stages of activity as well as those of healing can best be made by the Schilling count together with the subcutaneous tuberculin test.

4. The subcutaneous tuberculin test together with the Schilling count is the most delicate and most accurate method in the diagnosis of early manifest and induced focal activity.

On the basis of his findings, the validity of the author's classification (see below) originally proposed in 1929 is reaffirmed:

"*Active Tuberculosis.*—A patient who has a degenerative neutrophilic shift to the left with the Schilling blood differential, due to tuberculous infection, with a further shift to the left of the neutrophilic leukocytes or a drop in the lymphocytes following 10 mg. or less of Old Tuberculin.

"*Potentially Active Tuberculosis.*—A patient who has a normal or a degenerative neutrophilic shift in the Schilling differential, of doubtful origin, and who has an increased degenerative neutrophilic shift or a drop in lymphocytes following 10 mg. of Old Tuberculin. This is associated with a general tuberculin reaction with fever.

"*Apparently Healed.*—A patient who has a normal or a degenerative neutrophilic shift in the Schilling blood differential, of doubtful origin, and who has an increased degenerative neutrophilic shift or a drop in lymphocytes following 10 mg. or less of Old Tuberculin, not associated with a general tuberculin reaction with fever.

"*Healed Tuberculosis.*—A patient who has a normal or degenerative neutrophilic shift to the left in the Schilling blood differential of doubtful origin, and who fails to get any change in the Schilling blood differential following 10 mg. of Old Tuberculin."

MELANURIA, Blackberg, S. N., and Wanger, J. O. J. A. M. A. **100:** 334, 1933.

The following test for melanin is proposed as more reliable than those in common use:

1. A twenty-four hour specimen of urine is evaporated to one-fourth of the original volume.

2. One gram of potassium persulphate is added for each hundred cubic centimeters of the concentrated urine.

3. At the end of two hours, an equal volume of absolute methyl alcohol is added. The precipitated melanin is allowed to settle.

4. The precipitate is filtered off and washed with water till the washings are colorless, then washed with methyl alcohol, to remove any soluble pigments remaining. Finally, it is washed with ether. If the test is positive there remains on the filter paper a brownish black precipitate, which can be dissolved off with alkali, most conveniently with 5 per cent sodium hydroxide. Acidification of the alkaline solution causes a reprecipitation of the melanin.

ACETONE, Detection of in Urine, Leti, F. Chim. Ind. Agr. biol. **8:** 419, 1932.

Place 1 gm. of a mixture of 4 gm. of sodium nitroprusside and 10 gm. of *dry* sodium hydroxide in a porcelain dish and add 1 to 2 drops of urine. In the presence of acetone a red-lilac color at once changing to brown appears. In the absence of acetone the red-lilac color first appearing turns canary-yellow.

The reaction is said to respond to as little as 0.025 per cent of acetone.

BLOOD, Occult, Demonstration of, Mediriger, P. Deutsche Ztschr. ges. Gericht. Med. **20:** 74, 1932.

The following method is stated to be both delicate and reliable:

Reagents:

A. "Leucomalachite green"	1 gram
Glacial acetic acid	100 c.c.
Distilled water	150 c.c.

B. 1 per cent hydrogen peroxide.

Method.—One drop of a mixture of 8 parts of A to 2 of B is added to the material to be examined. In the presence of blood a dark green color appears within ten seconds turning to dark bluish-green in one minute.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

A Textbook of Pathology*

THOSE who are familiar with MacCallum's Pathology, and their name is legion, will welcome this fifth edition which has been extensively and thoroughly revised in accordance with the changes which have occurred, and the newer views now accepted in this field.

The plan and character of the book remain unchanged and based upon the conception that "all pathologic disturbances are the result of some form of injury or of the immediate or remote reactions of the body to injury."

The sections on tuberculosis, syphilis, endocarditis and disturbances of the organs of internal secretion have been revised in accordance with the newer conceptions of these subjects. New sections have been added, notably those upon infections of uncertain character, diseases due to injury of the organs of internal secretions, diseases due to specific dietary insufficiencies, disturbances of lipoid metabolism, and diseases of undetermined origin affecting bones. Hodgkin's disease is now discussed in a separate chapter.

While not intended as a book of reference the volume will undoubtedly, as before, be widely read and frequently consulted for its clear and logical presentation of the subject.

There is a full index and the typography and excellent reproduction of the equally excellent illustrations are deserving of the highest commendation.

The book can be recommended without reserve not only to the student and physician but also to the worker in this field desirous of refreshing his memory, either of the basic principles, or the newer conceptions in the field of pathology.

Dosage Tables of Roentgen Therapy†

THIS book should be particularly useful in clinics devoted to roentgen therapy. About one-half of the book consists of dosage tables, the first portion describing the methods by which the tables were compiled and the various factors entering into their construction.

The book is not to be taken as presenting, as it were, prescriptions for roentgen therapy, but is concerned mainly with a consideration and estimation of physical quantities which are presented in a concise and understandable manner. The depth dose through a portal of definite size, the effective dose, and the skin dose can all be readily determined, although some practice in the use of the tables will be necessary to eliminate probable sources of error.

Therapeutic Uses of Infra-Red Rays‡

ONE would gather from a perusal of this book that 90 per cent of the aches and pains of mankind can be relieved if not cured by infra-red rays. There may be some conditions which infra-red rays would cure but heat would not alleviate, but they are not to be found mentioned in this book.

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*A Textbook of Pathology. By W. G. MacCallum, M.D., Professor of Pathology and Bacteriology, The Johns Hopkins University, Baltimore, Md. Fifth Edition. Thoroughly Revised. 1212 pages with 652 illustrations. Philadelphia and London, 1932. W. B. Saunders Co.

†Dosage Tables of Roentgen Theraphy. By Frederich Voltz, Head of The Radiological Department, University Clinic for Women, Munich, Cloth, 120 pages, Oxford University Press.

‡Therapeutic Uses of Infra-Red Rays. By W. Annondall Troup M.C., M.B., Ch.B. Cloth, 57 pages, 16 plates, The Activic Press, Ltd.

The author details the cure of nearly every medical condition except the acute infections and the metabolic diseases, among them abscesses and bronchitis, carbuncles and cataracts, earache and epididymitis, frostbite and leukopenia, anterior poliomyelitis, toothache and writer's cramp.

The illustrations show various types of apparatus for the application of infra-red rays, apparently in every instance stock cuts bearing the manufacturer's name.

The opening paragraphs of the chapter on technic follow: "It is very important—indeed imperative—to have a spacious, bright, and well-ventilated room for treatment. I would suggest that the walls should if possible be distempered in primrose or pale blue and the curtains preferably blue."

This volume can hardly be recommended as a dispassionate or scientific presentation of the place of infra-red rays in the treatment of disease.

Sex and Internal Secretions*

AS STATED in the preface, this is not—nor was it intended to be—"a popular book on sex." Its purpose is "to survey the most important recent researches in the problems of sex, especially those concerned with internal secretions, in order that the concepts already established by experimental evidence may be clearly stated and readily available."

To this end, under the editorship of Dr. Allen, a group of investigators whose work has established them in their respective fields, have contributed in this volume a comprehensive and authoritative presentation of the many notable discoveries and factual contributions which have accumulated during the past decade in the realms of general biology, physiology, and psychology of sex.

It would be impossible within the limits of an ordinary book review to outline, even cursorily the volume and extent of the data discussed within this book. Some idea of its extent may be had from the table of contents which follows the general biologic introduction.

Interrelation of Genic and Endocrine Factors in Sex (C. H. Danforth); Genetics of Sex in Drosophilia (C. B. Bridges); Embryological Foundations of Sex in Vertebrates (B. H. Willier); Sex Derivations, Inversions, and Parabiosis (E. Witschi); Metabolism and Sex (O. Riddle); Biology of the Testis (C. R. Moore); Biochemistry and Assay of Testis Hormones (F. C. Koch); Ovarian Follicular Hormone, Theelin; Animal Reactions (E. Allen); Biochemistry of the Follicular Hormone, Theelin (E. A. Daisy); Physiology of the Corpus Luteum (F. L. Hisaw); The Mammary Glands (C. W. Turner); Plumage Tests in Birds (Domm, Juhn, and Gustavson); Ovulation, Transport, and Viability of Ova and Sperm in Female Genital Tract (C. G. Harman); Effect on the Reproductive System of Ablation and Implantation of the Anterior Hypophysis (P. E. Smith); Effects of Extracts of Anterior Pituitary and Similar Active Principles of Blood and Urine (E. T. Engle); Anterior Pituitary Changes Referable to the Reproductive Hormones and the Influence of the Thyroid and Adrenals on Genital Function (Severingham, Engle and Smith); Sexual Drive (C. P. Stone); Endocrine Disorders of Sex Function in Man (J. P. Pratt).

An extensive index of authors and of subjects is appended.

To those interested in the problems of sex, those engaged in investigation, and those casting about for promising problems of investigation this volume should prove invaluable.

The format is excellent and conforms to the motto of this house: "Sans Toche."

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*Sex and Internal Secretions, Edited by Edgar Allen, Professor of Anatomy, University of Missouri, Cloth, 951 pages, numerous tables and illustrations, Baltimore, Williams and Wilkins Co.

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EDITORIAL

The Serum Bilirubin Test

FEW laboratory tests have commanded so much attention as the so-called van den Bergh test for bilirubin. "So-called" is used advisedly, because the test, first published by van den Bergh and Snapper in 1913, was modified by van den Bergh and Muller three years later, and it was not until 1918 that van den Bergh published alone and then did not add materially to the technic. Indeed the test itself may be looked on as an application of one devised by Ehrlich years before in which he showed that when diazomium salts were added to bilirubin dissolved in chloroform or alcohol a red color was produced when the solution was neutral, or a bluish color when the solution was acid. Pröscher suggested the clinical use of the test a few years later. It would appear that McNee, the first to publish on the subject in English, was chiefly responsible for the term "van den Bergh test," for his paper contained the following:

"He (van den Bergh) virtually rediscovered the color reaction given by bilirubin with the diazo reagent of Ehrlich. An attempt is evident in recent German papers to claim the test now applied to the serum in jaundice under the name of Ehrlich-Pröscher reaction. There seems no doubt, however, that since van den Bergh first applied the reaction to the examination of blood serum, and has made such important original observations and deductions from its use, the test as applied to the study of jaundice should with fairness and right be known as the 'van den Bergh test.'"

In spite of this statement the test represents clearly the work of at least four men. How much better and fairer to all concerned it would have been to have called the test the "diazo bilirubin test." For few tests, has so large a number of modifications been offered as for this one, since van den Bergh and his coworkers announced it; nor can one name a test, as useful and yet one around which so many errors have gathered.

It would not be profitable to mention the numerous errors in bibliographic references to the test nor the many errors made in ascribing certain statements to various authors who did not make such statements. However, it is very much worth while to record certain fundamental errors, since these have a practical bearing on the interpretation of the test itself.

As a setting for the discussion, it is desirable to give briefly the outstanding events in the history of the test after Ehrlich's contribution of 1883. In 1913 van den Bergh and Snapper published the tests as applied to blood serum, and in spite of van den Bergh's later statement that the test was simply an "estimation" and not a "quantitative determination" of bilirubin, they published the method first under the subtitle of "Eine quantitative Bestimmung des Bilirubin im Blutserum," the details set forth clearly pointed to quantitative considerations although as the matter eventually worked out the test certainly warranted only an "estimation." This paper concerned itself with the amount of bilirubin in different sera, not only of man but of other animals. The amounts were expressed in terms of concentration, and these in turn were allocated to ten classes. In performing the test the investigators added alcohol to the serum, centrifuged, withdrew an aliquot part of the supernatant liquid, and then added the Ehrlich reagent. They mentioned the volume of the protein precipitate and the contraction of the total volume due to the retraction of the mixture of alcohol and serum. These two items were taken into account in making the computations, as is clearly shown in the example cited in the paper.

In 1916 van den Bergh and Muller called attention to the fact that two kinds of reactions in serum could be shown to exist according to whether the red color developed before or after the addition of alcohol; one reaction they referred to as direct and the other as indirect. They began the development of the idea that the direct reaction occurred in serum from patients with obstructive (mechanical) jaundice and the indirect reaction occurred in serum from patients with hemolytic jaundice, the latter also being the reaction of normal serum.

Van den Bergh, in his monograph of 1918, developed these ideas further and advanced two new procedures. In the first place he introduced an ether solution of iron rhodanate as a standard, and added 0.5 c.c. of alcohol after the first alcoholic precipitation and before adding the Ehrlich reagent. He also modified the calculation so as to ignore the volume of the precipitate, considering the serum as being diluted five times. He reported quantities under the term "Bilirubinwert." He placed his normal values as 1:400,000 to 1:250,000, or 0.5 to 0.8. In 1921 van den Bergh used the term "unit," and defined it as being the equivalent of 1:200,000 dilution of bilirubin. Since 1:200,000 really means 0.5 mg. per cent, one unit means 0.5 mg. per 100 c.c., although reporting on the basis of milligram values did not become popular until much later. In this paper van den Bergh gave as normal values 0.2 to 0.6 units, or 1:1,000,000 to 1:400,000.* Apparently therefore McKee is not responsible for the translation of "Bilirubinwert" to "bilirubin unit" as stated by Hunter, and the confusion caused by such a term as "unit" if such there be, must rest jointly on Botzian (1920), Rosenthal (1920), van den Bergh (1921), and Thannhauser and Andersen (1921). The last authors used the German term "Bilirubineinheit" in relation to the van den Bergh reaction to mean a value of 1:200,000, although Rosenthal, in 1920, also used the word. It was used, moreover, by Lepehne in 1921 and probably originated with Botzian, a pupil of Rosenthal. Hunter's objection to the term is not taken on altogether sound grounds.

Van den Bergh also described the delayed direct reaction as appearing after several minutes, and thought that the bilirubin which would produce a direct reaction had passed through the polygonal cells of the liver and had been taken out by the blood stream instead of being excreted through the hepatic ducts. The bilirubin giving an indirect reaction he thought to be bound with serum albumin.

Thannhauser and Andersen, believing that some of the bilirubin was precipitated in the albuminous precipitate, modified the technic. They added the Erhlich reagent first; then, after coupling had taken place (several minutes) they added alcohol and saturated solution of ammonium sulphate. They ignored the rather large colorless precipitate and heavy solution of ammonium sulphate. Their diazo reagent was five times the strength of the original reagent.

The matter of standards for comparison has received considerable attention. McNee and Keefer (1925), on the authority of van den Bergh and Muller, introduced a standard of cobalt sulphate; Rhamy and Adams, a solution of potassium permanganate; Hall added sulphuric acid to the cobalt sulphate, and White added hydrochloric acid to the same salt. Nichols and Jackson used cobalt chloride and hydrochloric acid. All these standards have been used in various colorimeters and in transverse comparison tubes.

Although numerous modifications of the test have appeared, it should be noted that from time to time authors have called attention to the fact that temperature, length of time elapsing after withdrawal of blood, and the P_H all have important bearing on the results, and that the natural evaporation of

*There is obviously an error in these figures since 1:400,000 is 0.5 units.

both alcohol and ether in the ethereal standards will greatly alter the values derived.

The mode of determining the type of reaction is also a question. Feigl and Querner early discovered that in some sera, after an initial development of color some further color developed, and the reaction was called biphasic. Unfortunately, Lepehne's modification in the form of a ring test has been generally overlooked, although it appears to be the most accurate method of determining the type of reaction present. Elton, a decade later, redescribed the ring test as an innovation. Various rules have been laid down concerning the time to wait for the appearance of the color; little agreement exists, although the general feeling is that a direct reaction occurs within thirty seconds and may or may not be biphasic, that if no color develops for five minutes the reaction is indirect, while a delayed direct reaction occurs between these limits of time.

The diazo color has been variously described. Most authors, including van den Bergh, have termed it red (rot), but many terms, such as red-violet, red-orange, red-blue, etc., have been used to describe the color produced. Peters and Van Slyke gave the color in prompt direct reactions as blue, and went to the trouble to italicize the word; yet in another place in the text they described the color as bluish-violet, and the color in the indirect reaction as red. It is true that the P_H affects the color, but in fresh serum giving a direct reaction with the original Ehrlich reagent, the color is distinctly red. If Thannhauser and Andersen's formula for the Ehrlich reagent is used, the color tends to be more violet or blue in direct reactions, but red in all other types.

The mathematics of the test has proved to be a stumblingblock. At the outset, van den Bergh and Snapper used a standard made as follows: 5 mg. of pure bilirubin was dissolved in 100 c.c. of chloroform, and 1 c.c. of this solution was evaporated to dryness; this, in turn, was redissolved in 10 c.c. of alkaline alcohol to which 2.5 c.c. of Ehrlich's reagent was added. Curiously enough they considered this a 1:200,000 solution instead of a 1:250,000 solution, as it actually was, and this error was carried down through all the literature until it was pointed out by Hunter in 1930, and even since then practically all authors have continued the error. Thus, it appears that all recorded determinations on this basis alone are 20 per cent too high, because all artificial standards, with the exception of those made by White, who profited by Hunter's discussion, have been made by comparison with this original formula.

The effect of the precipitate on the ultimate color, and hence on the final result, has been a matter of considerable controversy. In the original technic the volume of the precipitate was deducted from the total volume of the supernatant liquid, and was therefore taken into the calculation. Evidently, however, van den Bergh and Snapper forgot to take into consideration the dilution effected by the addition of 0.25 c.c. of Ehrlich's reagent, which in effect diluted their final solution 20 per cent. In 1918, van den Bergh corrected this error, but stated that the precipitate was not sufficiently great to

be considered, and thus his dilution was considered to be 1:5. It has been shown by subsequent authors that when alcohol only is used as a precipitating agent, the factor of dilution is approximately 1:4 although this varies with each individual test. Thannhauser and Andersen considered their dilution as being 1:6, and failed to take into consideration the rather large bottom layer which apparently contains little if any color. Subsequent authors, such as Hall, have not agreed with this position, and have found that the dilution is actually only about 1:3 by this technic. The most careful students have advised measuring the volume of the supernatant liquid and using this as the factor of dilution. Of authors of recent textbooks, this plan has been adopted by Hawk and Bergeim in the latest edition of their work, and by Trumper and Cantarow, but the volume of the uncolored bottom layer has been ignored by Peters and van Slyke and by Todd and Sanford, while a compromise of considering the dilution 1:4 is accepted by Harrison, Osgood and Haskins, Kolmer and Boerner, Gradwohl, and others.

Although it is of the utmost importance to know by how much the serum is diluted in order to make the correct calculation, it is also important to know whether some of the bilirubin or some of the color is carried down in the precipitate. Concerning these two points there still remains obscurity but on the basis of information available it would seem that the best calculation would be based on using the volume of the supernatant liquid as the factor of dilution, especially if Thannhauser and Andersen's method is followed. Many authors ascribe procedures to Thannhauser and Andersen which they did not use; thus, it is not uncommon to find authors recommending use of the weaker Ehrlich's reagent, changing both the amounts and the order in which reagents are added, and ignoring the fact that Thannhauser and Andersen used the ammonium sulphate only in cases of obstructive jaundice, with high content of bilirubin.

The error in the original standard, the usual errors in calculating the results, and the natural evaporation of the alcoholic solution have all tended to make too high values for serum bilirubin. With these errors varying as they do, there is no wonder that little agreement exists as to the normal range of concentration of serum bilirubin in man. The values as given range almost from infinitesimal amounts to the higher figure of 2 mg. per cent, as given by Greene and his coworkers. Obviously, for a correct understanding of such values, the exact technic and calculation must be clearly noted.

As important as the quantitative determination of bilirubin would appear, the type of reaction seems to be more important. It is quite clear that while van den Bergh's original statement that a direct reaction occurs in the presence of obstructive jaundice, and an indirect reaction occurs with the serum of normal persons and with that of patients with hemolytic icterus is true in general, it is equally true that this relationship does not always hold. Mann and Magath have clearly demonstrated the fact that bilirubin is formed outside of the liver, and this bilirubin gives an indirect reaction. There is considerable evidence to support the view that with the serum of man a direct reaction is not obtained unless the bilirubin has passed through the polygonal

cells of the liver, and in spite of the fact that the reactions are different in these types of bilirubin, Magath and Sheard, and others, have shown that the bilirubin itself is the same substance, or at least gives the same spectrophotometric curve and the same general reactions. Just how the bilirubin that gives the indirect reaction is bound up is not known, but it might be suggested as a logical view, that the bilirubin which reacts indirectly, is associated in some way with serum colloid, that it is colloidal in nature, that it is changed to a crystalloid form as it passes through the polygonal cells of the liver, and that this is the factor which converts the bilirubin into a state which yields a direct reaction.

In the light of this explanation and other known facts, the type of reaction becomes markedly significant and indicates that when a direct reaction is present there is injury to the polygonal cells in the liver. Contrary to the usual conception, one often sees direct reactions when the quantity of bilirubin in the blood is small, for the hepatic cells may have been definitely injured either by mechanical or physiologic obstruction. The emphasis in the literature, in the past, has been made on the type of jaundice present as evidenced by the type of reaction. In the light of present knowledge, this appears unfortunate, for the qualitative test is far more useful as an indication of hepatic injury than it is as an indication of the type of jaundice, many direct reactions being noted when there is no mechanical obstruction. A reason for missing these direct reactions when the total quantity of bilirubin is small is found in the usual method of performing the test, and may be avoided by making a ring test of the reaction on very fresh serum.

The quantitative determination of bilirubin has its greatest value in following the course of patients before and after operation or some other form of treatment, and a falling value for bilirubin often will indicate a good prognosis or a suitable time for surgical intervention before any other test or observation is definite enough for such a decision. On the other hand, a rising value for bilirubin should be considered with apprehension, and following an operation on the biliary tract indicates a grave prognosis.

It can be seen that there is still ample opportunity for scholarly investigation of serum bilirubin, permanent and more correct standards need to be provided, the effect of precipitating reagents needs to be studied, and the optimal conditions for development of color before and after addition of alcohol need to be more carefully examined. In spite of all these deficiencies, the test, if even reasonably well carried out, will yield extremely valuable information and must still be considered the most useful single laboratory test for hepatic function.

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—*T. B. M.*

CHECKING THROUGH*

EDWARD J. PODOLSKY, M.D., BROOKLYN, N. Y.

"WHY," said I to the detail man, "should I use your stuff when I can write just as good a prescription for nonproprietary items?"

"It's a matter of checking through," said he. "The care expended by a manufacturer in control of quality and uniformity means something to you and to your patients." Perhaps I did not look impressed, perhaps he was tired and the day hot—anyhow he added vehemently, "Go see for yourself, if you don't believe me."

That's how it started. I mentioned it to a certain medical editor. "Go," he told me, "to Bridgeport. There is a laboratory there that is outstanding and unusual. Don't ask for an appointment, just drop in and talk to them." So one bright morning I was greeted by Dr. E. C. Fanto, Chief of the McKesson and Robbins Research Laboratories, Dr. W. J. Horn, Mr. J. M. Leask and Dr. N. Michailovsky, his associates and their staff of six assistants.

"Checking through?" smiled Dr. Fanto, "of course we check through, but the work starts much earlier than that. As a matter of fact in this laboratory we have done work during the past few years that is of purely scientific or protective interest and from which we do not expect a return. Part of this has dealt with the possible effect of impurities in certain drugs on general well-being, and on the formation of tumors. Part of it has dealt with the action of drugs on tumors. Part of it has been toward the development of new and improved processes and methods. All of these things have cost probably over $100,000, which are a contribution to the scientific side of our work, a protection for the physician who prescribes and the patient who uses our products."

The laboratories occupy the entire fourth floor of the McKesson and Robbins plant in Bridgeport. Completed in 1930, these laboratories are supposed to represent the last word in equipment and in housing conditions for the animals used in the various controls and tests.

An unusual feature is the miniature manufacturing plant in which small test quantities of various medicaments may be made up under the same conditions that exist in actual commercial production. These are naturally quite often at variance with the conditions encountered in strictly laboratory work. Thus when a product is put into commercial production at the Bridgeport plant there are no uncertainties as to procedure or final perfection. Every step has been thoroughly worked out and tested in the miniature plant.

"No wonder you can do good work," I told Dr. Fanto, "with all this to work with."

*Publisher's Note: This article is being presented to give the readers of this journal an idea of the scientific work being done in the laboratories of modern pharmaceutical manufacturing firms and an appreciation of the scientific spirit pervading such institutions for the protection of physicians and their patients.

Fig. 1.—Method used for extracting the active principle from various drugs. The process is known as percolation, which is the extraction of soluble matter from its admixture of insoluble material by a suitable solvent. The solvent travels through the mass to be extracted, removing the partially saturated solution, from one surface while fresh solvent is admitted at the opposite surface.

Fluid extracts and concentrated extracts are prepared from the dilute percolate by evaporation, in a still, under reduced pressure to the desired volume.

This photograph shows the chemist watching and regulating the flow from the outlet of the percolator. This solution will then be transferred to the vacuum outfit shown on the left so that 1 c.c. of the finished extract will represent 1 gram by weight of the active principles of the drug.

"Well," he replied, "it is a help, but even before we had this plant our work was creative too."

I learned then that zinc stearate is one of the many products originated by McKesson and Robbins in their old plant and introduced by them to the medical and pharmaceutical professions. Another was the introduction of Albolene following the classical work of Sir Arbuthnot Lane on intestinal lubrication. This product is the finest obtainable mineral oil, put through the most exacting tests for viscosity and purity. These tests may be considered "checking through," but as I was told, the laboratory does not by any means stop there. Following the publication of certain experiments conducted at Manchester University in England, indicating a belief that impure mineral

F.g. 2.—This picture demonstrates how Medicinal White Mineral Oil (Albolene), is investigated in the McKesson and Robbins Laboratories, to conform to rigid specifications of this oil for internal use. It is standardized in regard to its lubricating properties expressed as the "viscosity" by measuring the relative degree of fluidity at 100° F. or the filling time in records of an empty graduated flask, 60 c.c. content, at a certain temperature to a certain mark. The behavior of this oil, when exposed to cold winter temperature, cooled down to 20° C., is examined. Note the freezing mixture to the right. The purity of the grade of refining of this oil is controlled by certain sensitive chemical reactions to be absolutely sure that the oil represents the highest degree of refining and not to contain any oxidizable, carbonizable substances or sulphur compounds.

oils possess carcinogenic properties, most careful checks were made on thousands of mice in the Bridgeport laboratories. In this work both external applications and certain forms of internal administration were employed. While the results have not been published it seems to have been established that the purest grade of mineral oil as typified by Albolene is harmless.

For years the laboratories of McKesson and Robbins have devoted themselves to development of new antiseptics, especially phenols with aliphatic radicals with a higher number of carbon atoms than previously, compounds of unusually high germicidal power and relatively low toxicity. At the an-

nual meeting of the American Pharmaceutical Association in 1929, there already was read a paper by L. Himebaugh and E. C. Fanto, on this subject. A series of comparative experiments comparing germicidal action with toxicity were presented. This study had been carried out on the living tissue of heart muscles of eight-day-old embryo chicks. They were able to point out at that time that one of the germicides in question, to which the laboratory

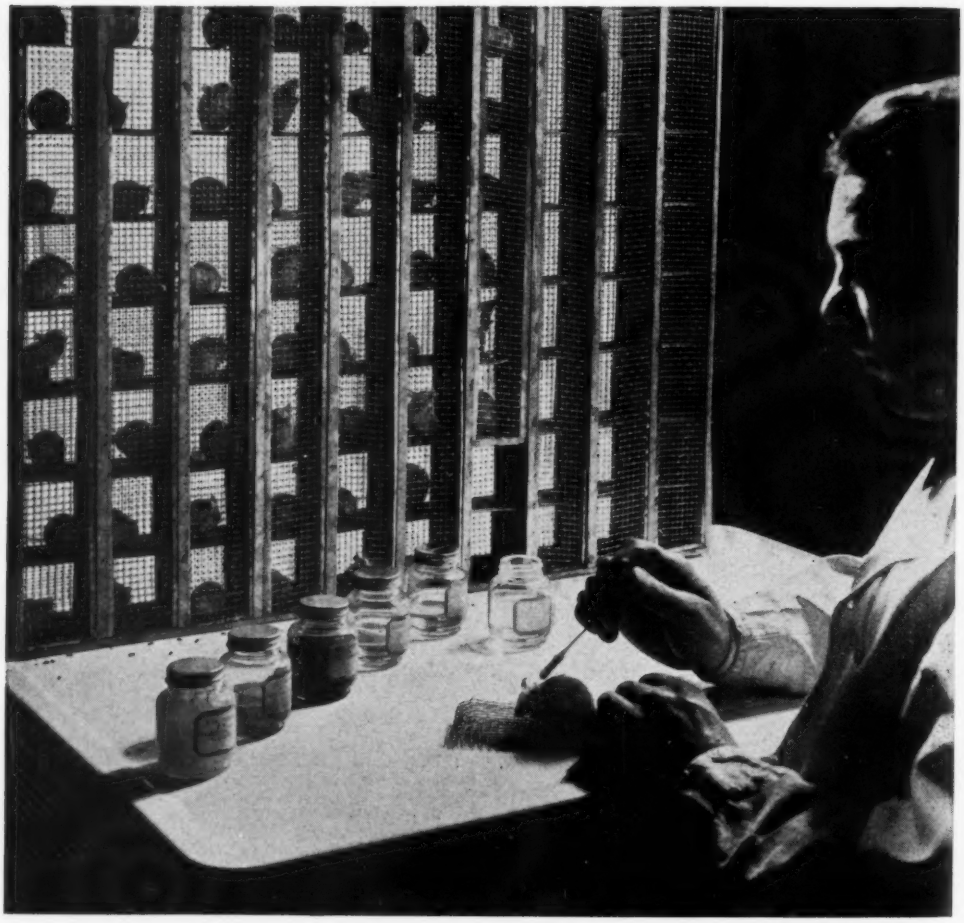

Fig. 3.—Investigation of certain properties of the chemicals used for preparation of compounds for external application is of great importance. Especially in view of the fact that some of the substances, when not sufficiently purified or improperly treated, may exert irritative or carcinogenic qualities in prolonged use. The picture shows skin painting by certain substances intended for external use and investigated for their effects through such application. These tests are often continued from 6 to 12 months before conclusions are reached.

number 801 was given, shows a tissue toxicity only 25 per cent of that of phenol. Taking into consideration the fact that this compound has a phenol coefficient of 533 against Staphylococcus Aureus, the toxicity to living tissue for a solution of equal germicidal power would be .004 per cent of that of phenol. Later tests on other animals have confirmed the relative nontoxicity of those compounds in the strengths of solutions as recommended for application.

We reached the vitamin assay laboratories under the supervision of Dr. W. J. Horn. Here lies the control for the McKesson and Robbins Cod Liver Oil and Cod Liver Oil Concentrate. Here, too, the check-up starts at the very source. To avoid variations, almost unavoidable in purchasing oils of different grades and origins on the open market, McKesson and Robbins acquired control of the largest Cod Liver Oil plant in the world, situated in Bergen, Norway. This plant now refines nearly one-quarter of the cod liver oil produced in Norway which leads the world in this commodity.

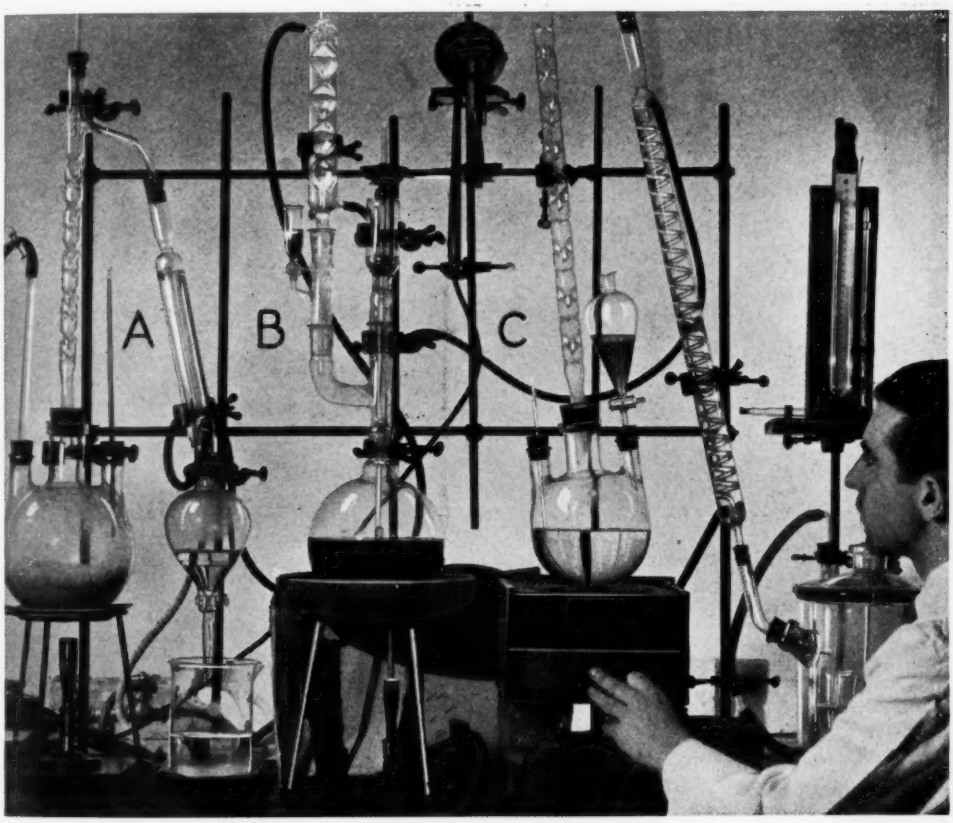

Fig. 4.—The original laboratory set-up for the Organic Synthesis of certain Phenolic Germicides, originated in McKesson and Robbins Research Laboratories.
Extensive research work is being done along the lines of producing powerful and very efficient antiseptics and germicides. The still A, at the left, represents the initial step, a destructive distillation, the product of which is then condensed in B, with other chemical compounds and the crude condensation product is finally purified by fractional distillation in C, under high vacuum.

The oil is produced under the most modern and scientific methods. The first step is to remove the livers, wash them and place them in clean covered double walled vessels through which steam is passed, all within a few hours of the time the fish is caught. This rapidity of handling eliminates unnecessary exposure to air which has the tendency to destroy Vitamin A. Next the oil is filtered into galvanized cooling tanks where it clears for 12 to 14 hours. Many refineries cease their work at this stage and ship the oil. Not so with McKesson's Oil. The crude oil is then pumped to refineries operated under a

vacuum system where the remaining blubber particles are removed and the product made nonfreezing by chilling it to 8° C. From the freezing tank the oil passes through a number of filter presses in a refrigerated room, where through an entirely new vacuum process the oil is clarified and all of the Vitamin A content retained. It is then shipped in tanks from which all air has been removed and replaced by nitrogen to prevent oxidation of vitamins in transit.

Fig. 5.—The technician in the bacteriological laboratory is about to start the Food and Drug Administration phenol coefficient test on a concentrated germicide of the coal tar type, (Lav-Ege). 0.5 c.c. of typhoid culture is added to one of a series of dilutions of this to determine which dilution kills the organism in ten minutes and not in five. This is found by making five-, ten-, and fifteen-minute transfers from the dilutions after inoculation into subculture tubes of broth, and incubating these for regrowth of the organisms. The result gives a coefficient of 3.8 against Eberthella typhi and 1.6 against Staphylococcus Aureus. This particular test is an example of one of the many kinds executed in the laboratory where bacteriological research work is being carried on continuously in an attempt to perfect even more efficient germicides than are now in common use.

Before shipment each lot is biologically tested under the supervision of Professor Poulson. When the oil is received by McKesson's it is again assayed biologically for Vitamins A and D according to the methods proposed by the vitamin committee of the A. D. M. A. McKesson's medicinal cod liver oil will average 800 units and never less than 500 units of Vitamin A and 150

units, never less than 100 units, Vitamin D per gram of oil when tested biologically by the above method.

"Concentrates?" I asked. "Liquid and tablets," smiled Dr. Horn. The liquid concentrate is made under the Marcus patent which ensures almost complete retention of the original vitamin content. It is intended primarily for infants and children where minims may be substituted for teaspoonfuls of whole cod liver oil. The tablets also prepared by exclusive methods, followed as a logical development and are standardized at a very high Vitamin A

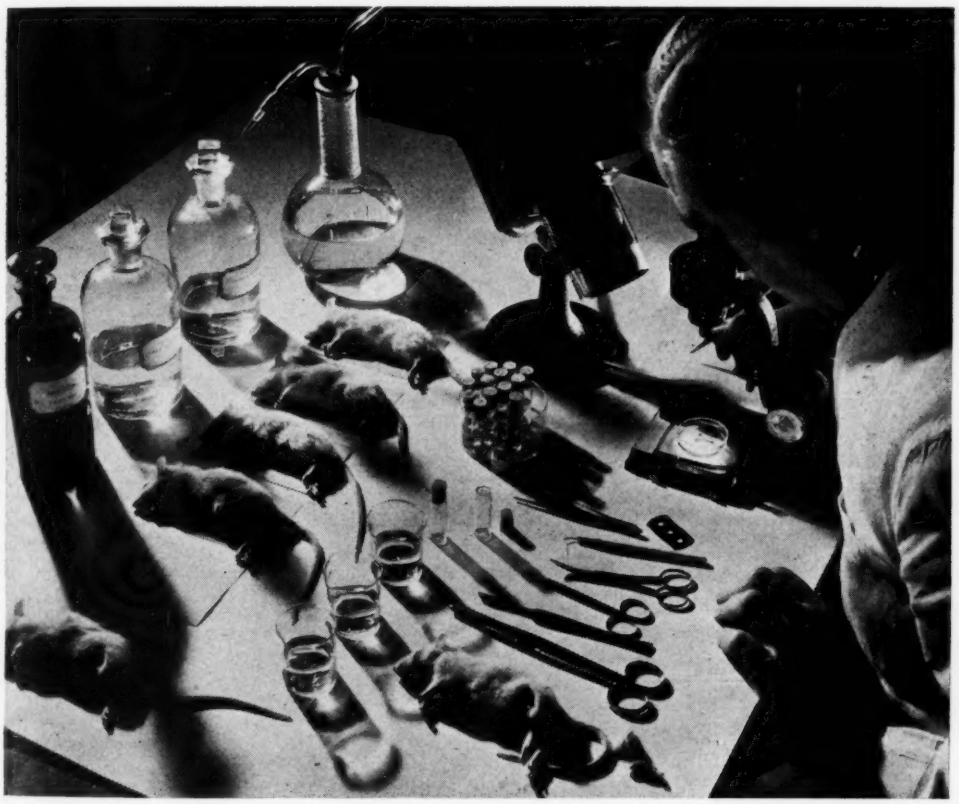

Fig. 6.—This photograph illustrates one of the stages in the biological assay of Vitamin D, the examination of the tibia of a rachitic rat after treatment with cod liver oil. Rigid control and accurate data are kept on each assay so as to be sure that each package going to the consumer contains the required potency as specified on the label. The A. D. M. A. methods of assay are used.

unitage (1000 units per tablet). In addition each tablet contains a definite proportion of di-calcium phosphate.

"What is your most recent development?" was my next question.

"Well," I was told, "that is hard to say. Here, as with other large pharmaceutical houses, we are constantly endeavoring to develop new ideas, products as well as technic."

I gathered for instance that at the present moment some quite extensive clinical investigation is being carried on to determine the merits of a colloidal

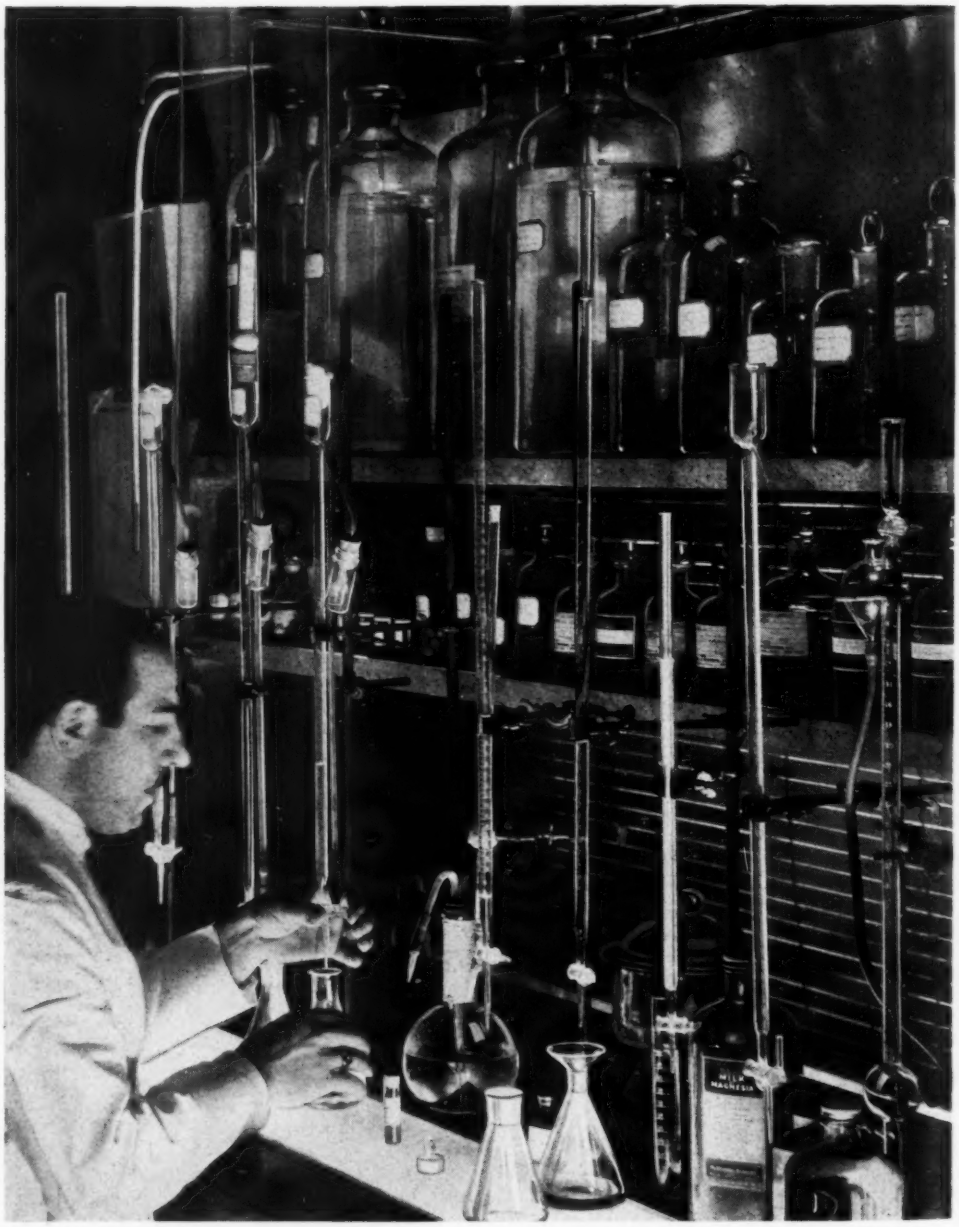

Fig. 7.—Volumetric Analysis. McKesson and Robbins pharmaceutical preparations are assayed according to the pharmaceutical standard methods. The picture records an up-to-date titration table, on which, by volumetric analysis, strength and purity are determined using Standard Volumetric Solutions and specific color indicators.

In actual operation, the solution of a proper reagent, whose exact strength at the time of the determination must be known, is carefully added from a burette, until the completed color change indicates the reaction. The chemist in this photograph is measuring a certain amount of liquid into an Erlenmeyer flask. From the volume of the solution of the reagent consumed to produce this reaction, the quantity of the substance sought is calculated.

copper morrhuate in certain tuberculosis cases. This product is given by injection intravenously or directly into the cavity. This work has been going on for over a year, but as yet the product has not been offered to the profession generally.

Then there are the experimental studies, laboratory and clinical, on the effects of certain chemical compounds on tumors. These have already lasted four years, and soon, I heard, a preliminary report will be made.

Even the so-called routine work of control and testing may lead to im-

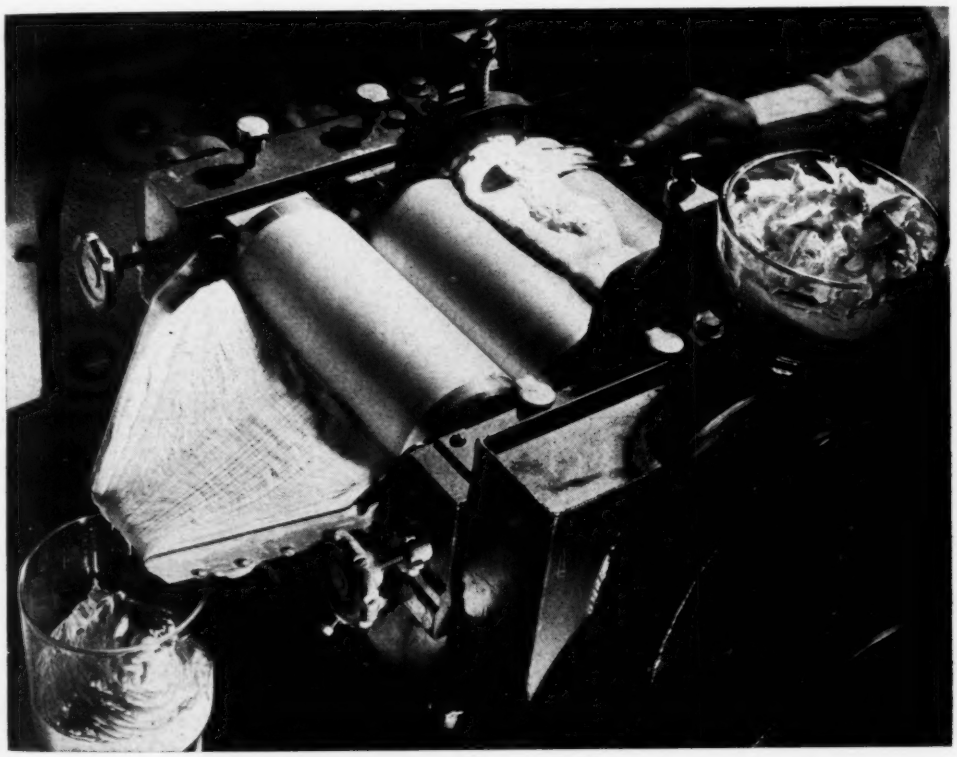

Fig. 8.—This picture represents a laboratory replica of ointment mass as used in a bigger scale in factory production of McKesson and Robbins. To prevent any gritty particles from being left in ointments, dentifrices, and salves these products are run through a system of rollers. The distance between the rollers is microscopically close so that the stream of ointment mass is compelled to pass through the rollers in a very fine layer.

proved products, new "developments" if you wish. In any event every batch of even the most "ordinary" type of pharmaceutical product goes through most careful testing processes, and, of course, each batch is numbered, listed and control samples kept.

Probably the last product to be completely developed in these laboratories is the McKesson and Robbins Copper Iron Compound. When interest in this type of medication developed, work was undertaken to find a preparation which would have a marked effect upon hemoglobin regeneration and which would be well tolerated and effective when administered orally. Realizing that

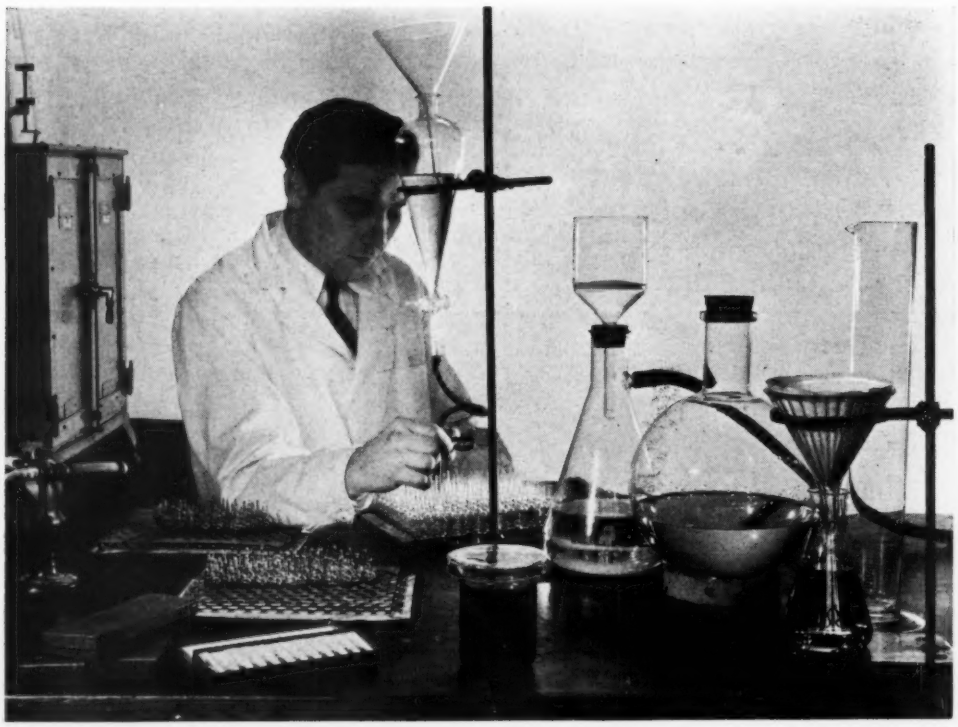

Fig. 9.—Developing a compound for any purpose requires a thorough study from different angles. It goes a long way before the final formula is reached. This photograph shows the process of filling the ampules, prior to their sterilization, with a compound for the laboratory investigation and experimentation on animals.

Fig. 10.—The importance of a thorough investigation of the effects produced by various chemical substances which, prior to human application, are studied on animals, leads us to a necessity of a very accurate analysis of the effects produced. Pathological examination of changes in animal tissues due to the external or internal application of certain substances represents one of the methods by which the effects are studied. The picture shows preparation of the tissue sections for the microscopical examination, in connection with the experimental work shown in Fig. 3.

inorganic salts of both copper and iron are astringent and not well tolerated by the digestive processes investigations were made using combinations of these two metals in the form of organic proteid compounds in which the copper and iron were in the "masked form." Many experiments conducted upon animals showed these combinations to be nonastringent or toxic and to cause no dis-

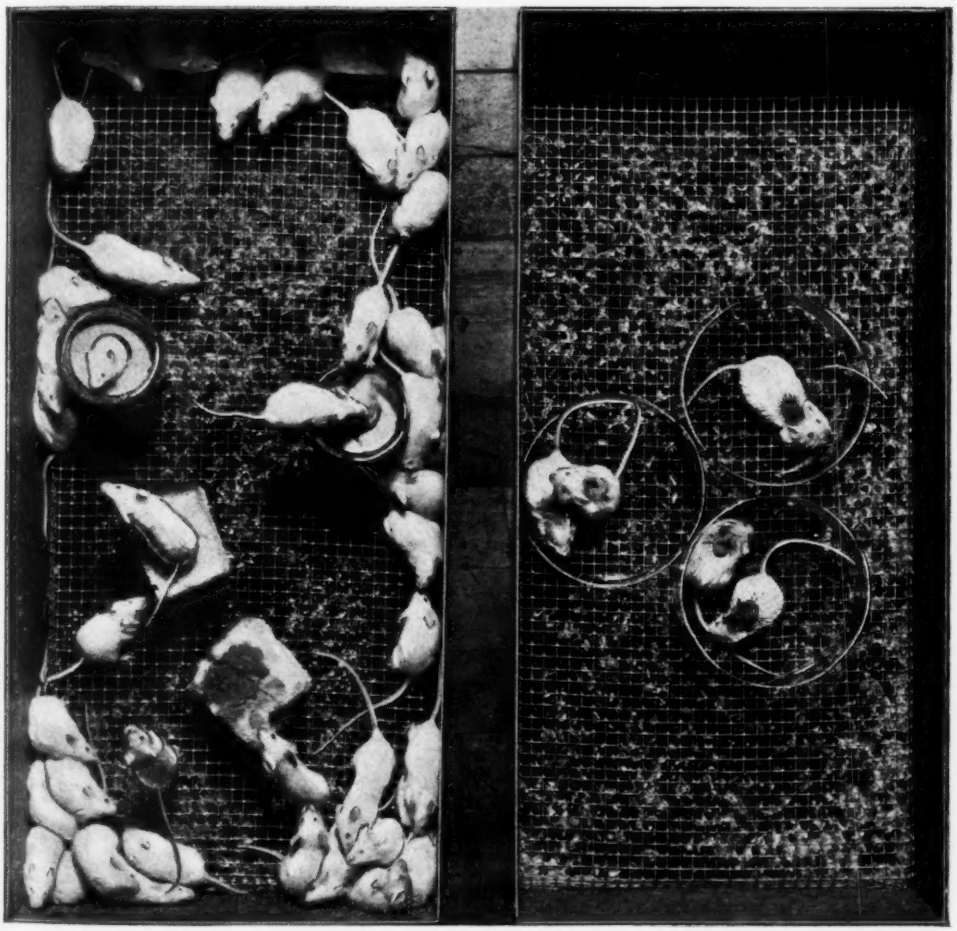

Fig. 11.—Animal experimentation plays a very important part in the study and development of certain preparations. This photograph offers an example of comparative toxic action produced by a highly refined product and the same product in an inferior state of purification. In the right cage it will be seen that only five mice are living after a three-month test period in which the inferior product was given internally. The left cage shows the large number of mice that were given the superior product, still living. Both cages contained the same number of animals at the beginning of the experiment.

turbances in the digestive processes. The effect upon hemoglobin regeneration in animals produced by the above combinations of this sort were found to be startling. In every series of experiments similar results were obtained. This work was then followed by clinical tests upon humans with equally gratifying results.

Each lot of *Copper-Iron Compound* is carefully standardized as to copper and iron content, the ingredients used in the manufacturing of the preparation being of the highest quality and purity obtainable.

And therewith closed my visit.

To me is was full of human interest and a revelation of the extent to which the important pharmaceutical manufacturers work for and with the

Fig. 12.—The chemical and physical constants of McKesson and Robbins' Essential Oils, Balsams, Oleo-Resins, and Vegetable Oils are thoroughly investigated. All samples are carefully examined for the presence of adulterants and other objectionable ingredients. All U. S. P. and N. F. Oils must meet the specifications given in these official publications. Determination of the optical rotation of a sample of essential oil with the polariscope is standard practice in these laboratories.

medical profession, of the amount of nonremunerative research work they do, and of the scientific accuracy of their methods. It is for these reasons that I have offered it for publication, supported by the excellent descriptive illustrations for which I am indebted to McKesson and Robbins.

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